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THE INHERITANCE OF PARTIAL THIAMINE DEFICIENCY IN LENZITES TRABEA [1]

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INTRODUCTION

The biochemical characteristics of some of the Ascomycetes, especially the yeasts (*9*)[2], *Neurospora* (*3*), and mutations of the penicillia (*4*), have been studied from the standpoint of genetics, but such studies among the higher Basidiomycetes are rare. In the Basidiomycetes the sporophore usually arises from diploid mycelium. The basidiospores, as a result of the reduction division, have but one haploid nucleus, and on germination give rise to haploid mycelium. It would be expected, therefore, that single-spore isolates from a diploid fruit body would reflect the biochemical and cultural characteristics of the original haploid parents. The amount of divergence among the single-spore isolates would be expected to depend upon the genetic factors influencing the characteristics under study. The mode of inheritance, whether simple or complex, would depend upon the number of factors involved and the complexity of their interaction. In the Basidiomycetes the expression of these genetic factors in the haploid mycelium and in the diploid mycelium (each cell of which contains two haploid nuclei of opposite sexual tendencies) may be studied under identical conditions. This is an advantage not afforded by the Ascomycetes or the Phycomycetes.

Growth factor and amino acid deficiencies are among the biochemical characteristics frequently studied. Fungi are self-sufficient, partially deficient, or totally deficient with respect to vitamins or growth factors. A fungus partially deficient for a given vitamin is unable to synthesize the vitamin at a rate adequate for the maximum rate of growth. The addition of an exogenous supply of the vitamin in question increases the rate of growth of a partially deficient fungus, and sometimes it also increases the amount of growth. Isolates of the same species have been found which had different degrees of partial deficiency (*11*).

Lenzites trabea Fries is partially deficient in thiamine (*8*). The haploid and diploid[3] cultures used in this work were obtained or derived

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[2] Italic numbers in parentheses refer to Literature Cited, p. 300.

[3] In this paper the terms haploid mycelium *or haplont* and diploid mycelium *or diplont* are employed in accordance with Buller's (*5*) usage.

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from a single fruit body. The paper reports the variation found in degree of partial thiamine deficiency of these cultures.

MATERIALS AND METHODS

A single fruit body of *Lenzites trabea* was collected in 1945 from an oak log at Morgantown, W. Va., and some 30 single-spore isolates were obtained from it. These isolates were obtained by allowing spores from the original fruit body to fall upon an agar plate and to germinate. The germinating spores were then picked out under the low power of a compound microscope, using the eye of a specially prepared small sewing needle. These isolates were allowed to grow, and the absence of clamp connections was verified by microscopic examination. These original single-spore haplonts were designated by the numbers 1 to 30.

The haplonts were paired to determine the compatibility group to which each belonged. By pairing two haplonts of different compatibility groups, the diplont (diploid mycelium producing clamp connections) was obtained. Thus, the symbol 3×4 designates the diplont derived from haplonts 3 and 4. As found by Mounce and Macrae (*10*), the single-spore isolates could be divided into two groups which were cross-compatible and mutually incompatible.

In most instances the diplonts fruited and produced viable spores. From these diploid fruit bodies, single-spore isolates were made. These F_1 haplonts were designated by letters following the symbol for the parent diplont, e. g., (3×4) B. Some haplonts formed hapliod fruit bodies (*2*) and produced viable spores; such single-spore isolates were designated by letters following the number of the parent haplonts, e. g., 7 B, etc.

The cultural studies were made in a liquid medium of the following composition:

Glucose	grams	25
KH_2PO_4	do	1
$MgSO_4 \cdot 7H_2O$	do	.5
Casein hydrolysate equivalent to	2 gm. of casein	
Fumaric acid	grams	1.32
Na_2CO_3	do	1.1
Fe^{+++}	milligrams	.2
Zn^{++}	do	.2
Mn^{++}	do	.1
Double distilled water to make	milliliters	1,000

The details and the method of preparing this medium in essentially vitamin-free conditions are given by Leonian and Lilly (*7*).

The pH of the medium at the time of inoculation was 5.5. The culture vessels used were 250-milliliter pyrex Erlenmeyer flasks, and 25 milliliters of medium was added to each flask and sterilized at 15 pounds' steam pressure for 15 minutes. Thiamine was added to half of the flasks before autoclaving. Inoculation was made by adding a 5-millimeter agar disk cut from the edge of an actively growing culture to each flask. The temperature of incubation was 25±1°C.

Two series of 14 flasks each were used to culture each isolate used. The first series contained no added thiamine; to the second thiamine was added at the rate of 2.5 μg. per flask. The growth of *Lenzites trabea* under the conditions of this experiment was slow; but not as slow as that reported for *L. saepiaria* by Fries (*6*), who found

that incubation periods in excess of 40 days were necessary to demonstrate partial thiamine deficiency. Two flasks from each series were harvested at intervals ranging from 7 to 10 days. The mycelium was dried to constant weight at 85°C. The data obtained in this way revealed the rate and the amount of growth of the culture over a period of about 55 days. Each datum in the tables is the average of two determinations. In some instances the pH of the culture medium was determined at the time of harvest.

EXPERIMENTAL RESULTS

GROWTH CURVES AND PH CHANGES IN THE CULTURE MEDIUM FOR REPRESENTATIVE SINGLE-SPORE ISOLATES

The extensive data obtained in this study will not be presented in full. Instead, the growth curves for the two haplonts exhibiting the extremes in partial thiamine deficiency will be given. The other haplonts derived from the original fruit body were intermediate in degree of partial thiamine deficiency; only the critical data for these isolates will be presented.

Figure 1 shows the rate and amount of growth and the change in pH of the culture filtrates of isolate 18 over a period of 56 days. In the absence of added thiamine the average weight of mycelium per culture at 56 days was less than one-third that attained in the presence of added thiamine after 22 days of incubation. It is probable that the slow rate of growth of this isolate in the absence of added thiamine would have continued for some time. The trend of the pH of the culture filtrates was downward at first, and when thiamine was not added this downward trend continued until the end of the experiment.

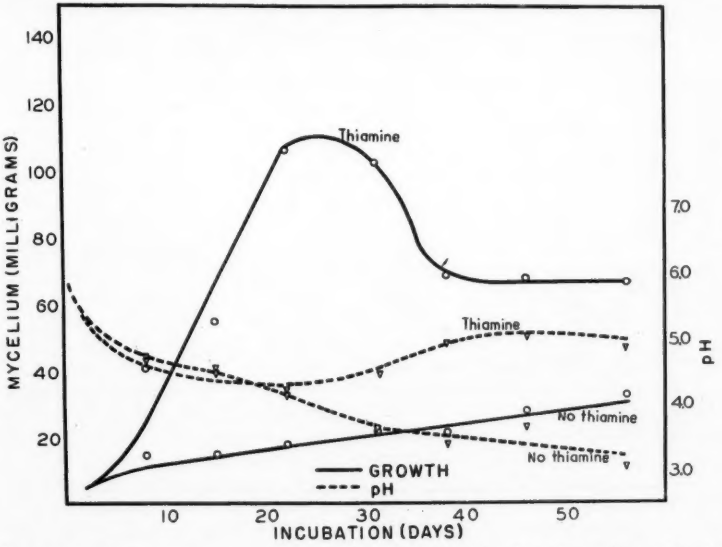

FIGURE 1.—Rate and amount of growth and change in pH of the culture filtrates of isolate 18 over a period of 56 days with and without the addition of thiamine.

The pH of the culture filtrates of the thiamine series fell until near the time that maximum weight of the mycelium was attained; thereafter the pH increased. The weight of the mycelium began to decrease soon after the attainment of maximum weight. The loss of weight due to autolysis frequently amounted to 50 percent of the maximum weight. *This loss of weight was* accompanied by higher pH values of the culture filtrates.

Figure 2 shows the rate and amount of growth and the change in pH of the culture filtrates of isolate 30 over a period of 56 days. No significant difference in the maximum weight of mycelium resulted when thiamine was added to the medium. However, there was a significant difference in the time of incubation required for isolate 30 to attain maximum weight under the two conditions. The pH of the culture filtrates of both series—those to which thiamine was added and those receiving no thiamine—fell during early growth, and then changed to higher values about the time the weight of the mycelium was at a maximum. Autolysis of the mycelium in both series followed the period when the mycelium attained maximum weight. The same sequence of events occured when thiamine was absent as when it was present; the only difference noted was that the rate of growth in the absence of added thiamine was less than in its presence. Thus maximum weight was attained after 22 days of incubation when added thiamine was present, and after 38 days in the absence of added thiamine.

It is assumed that the different rates of growth of these two isolates—18 and 30—in the absence of added thiamine reflect the varying capaci-

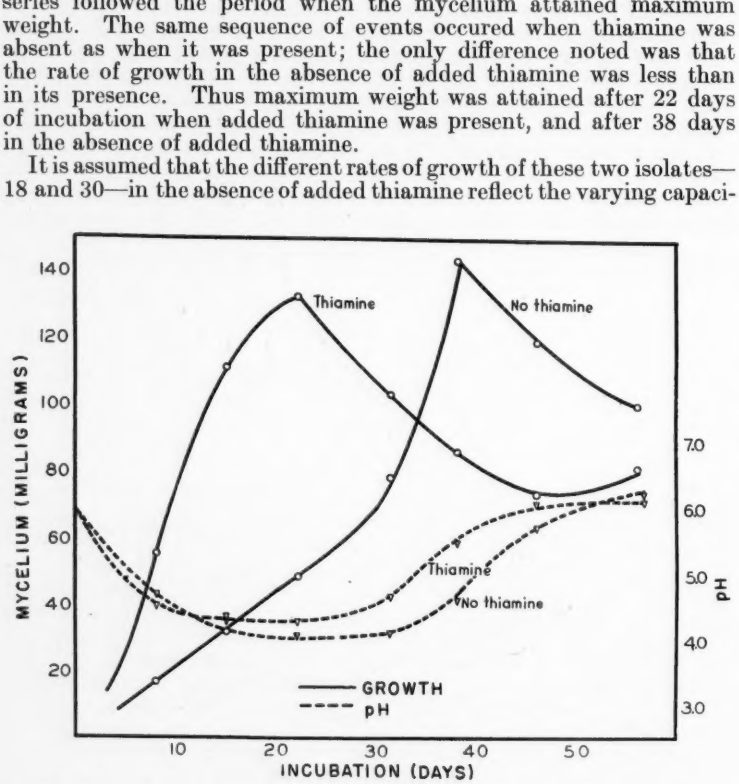

FIGURE 2.—Rate and amount of growth and change in pH of the culture filtrates of isolate 30 over a period of 56 days with and without the addition of thiamine.

ties of these isolates to synthesize thiamine. This interpretation is supported by the fact that in the presence of added thiamine both isolates attained maximum weight in about the same time. Isolate 18 was unable to synthesize thiamine as rapidly as isolate 30. The weight of mycelium produced by isolate 18 in the absence of added thiamine after 38 days of incubation was 22 milligrams, whereas that produced by isolate 30 during the same period was 144 milligrams.

In the experiments discussed in subsequent sections the average daily increase in weight of mycelium (milligrams-divided-by-days) was used as a measure of relative partial thiamine deficiency. The "days of incubation" used in this ratio was the time of incubation required to produce the maximum weight of mycelium. In some instances the maximum weight of mycelium was not produced by the end of the experiment; in such instances the ratios (milligrams-divided-by-days) were calculated from the weight of mycelium produced at the last harvest. This criterion was chosen because it relates both the amount of mycelium produced and the time required to produce it.

It seems obvious that when different periods of incubation are required by two isolates to produce the same weight of mycelium, the isolate having the shorter period of incubation has the greater ability to synthesize thiamine. Similarly, if one isolate produces a greater weight of mycelium than another isolate in the same period of incubation, the first isolate has the greater capacity to synthesize thiamine. The ratio used (milligrams-divided-by-days) places the isolates correctly with regard to degree of partial thiamine deficiency. The maximum weight of mycelium, rather than the weight of mycelium at any fixed time of incubation was used because of an intrinsic difference in the rate of growth of the various cultures.

RELATIVE PARTIAL THIAMINE DEFICIENCY OF ORIGINAL HAPLONTS

Fourteen of the single-spore isolates from the original fruit body were tested for relative ability to grow in the absence of added thiamine. At the same time these isolates were cultured in the same medium to which had been added 2.5 μg. of thiamine per flask. Fourteen flasks of each series were used for each isolate; seven harvests were made at stated times, the last on the fifty-sixth day of incubation. Since the data so obtained are too extensive to report in detail, the following data for each isolate are given: The days of incubation required for the isolate to attain maximum weight in the absence and in the presence of added thiamine, the maximum weight of mycelium produced, and the calculated average daily increase in weight in milligrams.

Three isolates (Nos. 4, 18, and 24) grew so slowly without the addition of thiamine that maximum weight was not attained after 56 days of incubation. The weight of mycelium given for these isolates in table 1 is that found on the fifty-sixth day. The calculated daily rate of growth should, however, be considered a fair approximation of their ability to synthesize thiamine.

The data in table 1 reveal a great difference in the ability of these 14 single-spore isolates to grow when no thiamine was added. Without the addition of thiamine the average daily increment in weight

ranged from 0.96 milligram for isolate 14 to 4.6 milligram for isolate 7, and if we include those isolates that did not attain maximum weight within 56 days, the extremes are 0.59 and 4.60 milligrams. The maximum weight of mycelium produced by these isolates (disregarding the 3 that did not attain maximum weight) ranged from 46 to 144 milligrams.

When thiamine was added the growth of all of these isolates was much more nearly uniform (table 1). The maximum weights of mycelium produced by these 14 isolates in the presence of added thiamine ranged from 104 to 140 milligrams. The average maximum weight was 126 milligrams. Only three isolates deviated more than ±15 milligrams from the average. In the presence of added thiamine, the average daily increment in weight ranged from 3.5 to 6.9 milligrams.

TABLE 1.—*Relative partial thiamine deficiency of 14 single-spore isolates of Lenzites trabea, derived from the same fruit body*

Isolate No.	No thiamine added			2.5 μg. of thiamine per flask added		
	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligrams/days)	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligrams/days)
	Days	*Milligrams*	*Milligrams*	*Days*	*Milligrams*	*Milligrams*
18	[1] 56	33	0.59	22	107	4.86
24	[1] 56	39	.70	22	119	5.41
4	[1] 56	48	.86	22	134	6.09
14	48	46	.96	38	140	3.68
5	48	69	1.44	30	136	4.53
17	48	79	1.65	22	110	5.00
10	31	53	1.71	38	134	3.53
20	46	84	1.83	22	133	6.05
23	46	101	2.20	31	125	4.03
28	31	69	2.23	15	104	6.93
3	38	96	2.53	22	110	5.00
9	38	99	2.61	22	139	6.32
30	38	144	3.79	22	133	6.05
7	30	138	4.60	22	138	6.27

[1] Maximum weight not attained by end of experiment.

RELATIVE PARTIAL THIAMINE DEFICIENCY OF DIPLONTS

The partial thiamine deficiency of diplonts derived from haplonts of known partial thiamine deficiency was studied.

The relative partial thiamine deficiencies were determined in the usual way. The data are given in table 2. In an essentially thiamine-free medium the average daily increments in weight of the cultures had a range of slightly greater than twofold. This is less than half the variation found among the haplonts. (See table 1.)

The diplont 4 × 28 in general resembled haplont 4, which made relatively poor growth in the absence of added thiamine. Diplont 4 × 3 also had a slow rate of growth. Both, haplonts 3 and 28 made good growth in thiamine-free medium. Only one diplont, 4 × 14, made more growth in thiamine-free medium than either parent haplont. This behavior is apparently exceptional, as the diplonts studied generally showed intermediate or low ability to synthesize thiamine.

Among the diplonts of which isolate 3 was a parent haplont, it was

noted that even when both of the parent haplonts made good growth in the absence of added thiamine (Nos. 7, 9, and 30), the average daily increment in weight was less than that of either of the parent haplonts. In no diplont studied involving isolate 3 was the ability to grow in the absence of added thiamine found to be greater than that of its more self-sufficient parent haplont. It is apparent that one nucleus modifies the action of the other, and this modification is toward lessened ability to grow in the absence of added thiamine. From these data and others, it appears that the influence of one nucleus upon another in diploid mycelium is complex.

In the presence of added thiamine the rate and amount of growth of these diplonts resembled that of the haplonts studied. The maximum weights ranged from 107 to 155 milligrams; the average was 139 milligrams. The average daily increases in weight ranged from 5.27 to 7.12 milligrams per day among the isolates.

TABLE 2.—*Relative partial thiamine deficiency of Lenzites trabea diplonts derived from haplonts of known partial thiamine deficiency*

Isolate	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium milligram /days)
	Days	*Milligrams*	*Milligrams*
4×28	[1] 64	57	0.89
4×3	48	55	1.15
4×14	48	80	1.67
3×4	48	55	1.15
3×8	50	65	1.30
3×9	48	68	1.42
3×7	46	71	1.54
3×30	42	84	2.00
28×18	42	46	1.10
125 [2]	50	72	1.44

[1] Maximum weight not attained.
[2] Tissue culture from original fruit body.

VARIABILITY OF THE FUNGUS

The wide variability among the original single-spore isolates both in amount and rate of growth was established early in this work (table 1). It was necessary to establish the degree of reproducibility of these experiments before any conclusions concerning inheritance could be reached.

The first method used to determine the amount of "normal" variation consisted of repeating the determination of relative thiamine deficiency for the same isolates several times, and this was done in a number of instances. None of these experiments for any one isolate was made at the same time. The data, which are presented in table 3, show considerable variation in growth rates when determinations were made at different times. In spite of the variations, the behavior of the different isolates was sufficiently uniform to prevent isolates 7 or 3 from being mistaken for isolates 4 or 14, but not sufficiently good to distinguish isolates 4 and 14.

Some of the cultures, after being kept on agar for some months, began to change with respect to partial thiamine deficiency. In general, this change was accompanied by a change in color of the mycelium and by the rate of growth in the presence of added thiamine. Such colored forms were studied separately. Other cultures appeared to be quite stable with respect to rate of growth and degree of partial thiamine deficiency.

The problem of "normal" variation was approached in another way. Single-spore isolates were made from haploid fruit bodies, and the partial thiamine deficiency of these was determined. All such isolates should be alike (excluding mutation). Isolates 7 and 18 were chosen for this experiment because they formed haploid fruit bodies readily and differed greatly in degree of partial thiamine deficiency.

All the single-spore isolates from haplont 7 attained maximum weight before the end of the experiment, and in this respect they resembled the haploid parent. All of these isolates were similar but not identical in degree of partial thiamine deficiency. The maximum weights of mycelium of the 10 single-spore isolates studied were: 89, 107, 95, 96, 89, 90, 105, 97, 109, and 91 mg.; the average maximum weight was 97 milligrams. The milligrams-divided-by-days ratios were: 3.18, 3.05, 2.26, 2.28, 2.12, 2.56, 2.50, 2.75, 3.89, and 2.59; the average was 2.72.

TABLE 3.—*Partial thiamine deficiency of 4 single-spore isolates of Lenzites trabea, as determined at different times*

Isolate No.	Date of inoculation	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligrams/days)	Average growth rate
		Days	*Milligrams*	*Milligrams*	
7	Jan. 6, 1946	30	107	3.57	3.99
	Mar. 20, 1946	30	138	4.60	
	Oct. 7, 1946	30	114	3.80	
3	Feb. 27, 1946	45	114	2.53	2.45
	May 6, 1946	38	96	2.53	
	Oct. 7, 1946	46	105	2.28	
4	Mar. 1, 1946	[1] 45	49	1.09	.91
	May 8, 1946	[1] 58	48	.83	
	June 11, 1946	[1] 58	47	.81	
14	Mar. 24, 1946	[1] 44	46	1.05	1.20
	June 11, 1946	[1] 58	78	1.34	

[1] Maximum weight not attained by end of experiment.

In general, the single-spore isolates of haplont 7 were not as self-sufficient as the parent haplont. Both the amount of mycelium produced and the daily increase in weight were less (compare table 3). The cause of this divergence is unknown.

Nine single-spore isolates from haplont 18 were tested for partial thiamine deficiency. None of these isolates attained maximum growth by the end of the experiment (59 days), and in this they resembled the parent haplont (table 1). At harvest on the fifty-ninth day the weights were: 23, 34, 18, 13, 11, 18, 19, 18, and 21 milligrams; the average weight was 18 milligrams. The milligrams-divided-by-day ratios were calculated from the data obtained at the last harvest. The values were: 0.39, 0.58, 0.37, 0.22, 0.18, 0.31, 0.32, 0.31, and 0.36,

and the average was 0.30. These values are somewhat less than those previously found for isolate 18 (table 1).

The most important conclusion to be drawn from these experiments is that single-spore isolates of haplonts all resemble the original haplont in respect to degree of partial thiamin-deficiency. This is in contrast to the wide spectrum of partial thiamine deficiences found among single-spore isolates derived from diploid fruit bodies (tables 1, 4, 5, and 6).

MODE OF INHERITANCE OF PARTIAL THIAMINE DEFICIENCY

From the data obtained in the foregoing experiments, it appeared likely that the inheritance of partial thiamine deficiency was complex. Supporting evidence was then obtained by studying the degree of partial thiamine deficiency of F_1 haplonts derived from diplonts of known haplont parentage. Three diplonts which fruited readily, 4×14, 3×4, and 3×7, were chosen.

TABLE 4.—*Relative thiamine deficiency of F_1 haplonts of Lenzites trabea derived from the cross 4 × 14*

Isolate	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligram/days)
	Days	*Milligrams*	*Milligrams*
4	[1] 58	48	0.83
14	[1] 58	83	1.43
(4 × 14) C	[1] 58	68	1.17
(4 × 14) T	48	84	1.75
(4 × 14) E	48	86	1.79
(4 × 14) G	38	85	2.24
(4 × 14) J	38	86	2.26
(4 × 14) O	38	108	2.84
(4 × 14) F	30	93	3.10
(4 × 14) B	30	112	3.73
(4 × 14) N	30	117	3.90

[1] Maximum weight not attained by end of experiment.

TABLE 5.—*Relative thiamine deficiency of F_1 haplonts of Lenzites trabea derived from the cross 3 × 4*

Isolate	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligram/days)
	Days	*Milligrams*	*Milligrams*
3	38	96	2.53
4	[1] 58	47	.81
(3 × 4) F	[1] 58	60	1.03
(3 × 4) I	[1] 58	70	1.21
(3 × 4) G	[1] 58	85	1.47
(3 × 4) C	48	78	1.62
(3 × 4) J	48	78	1.62
(3 × 4) K	48	79	1.65
(3 × 4) M	[1] 58	98	1.69
(3 × 4) H	38	69	1.82
(3 × 4) O	48	90	1.87
(3 × 4) D	48	104	2.17
(3 × 4) B	38	99	2.61
(3 × 4) L	30	116	3.87

[1] Maximum weight not attained by end of experiment.

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TABLE 6.—*Relative thiamine deficiency of the F_1 haplonts of Lenzites trabea derived from the cross 3 × 7*

Isolate	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligram/days)
	Days	*Milligrams*	*Milligrams*
3	46	105	2.28
7	30	138	4.60
(3 × 7)D	[1] 56	67	1.20
(3 × 7)C	38	100	2.63
(3 × 7)H	38	104	2.74
(3 × 7)L	30	94	3.13
(3 × 7)E	38	125	3.29
(3 × 7)B	30	106	3.53
(3 × 7)F	38	134	3.53

[1] Maximum weight not attained by end of experiment.

The F_1 haplonts of the diplont 4 × 14 were investigated first. Both of the haploid parents (4 and 14) made relatively poor growth in thiamine-free medium (tables 1 and 3). The data in table 4 show that the F_1 haplonts of the cross 4 × 14 varied greatly in degree of partial thiamine deficiency. While the amount of variation was somewhat less than that found for the original single-spore isolates, the range of daily increments in weight among these isolates was three and one-third fold. This variation is much greater than that of the original haploid parents. Four of the F_1 haplonts had a daily rate of growth more than twice that of either of the original haploid parents. These F_1 haplonts from the cross 4 × 14 made the expected amount of growth in the presence of added thiamine.

In the second experiment the F_1 haplonts obtained from the cross 3 × 4 were studied. The average daily increment in weight of haplont 3 was more than twice that of haplont 4 (table 3). If the degree of partial thiamine deficiency is controlled by a single gene, these F_1 haplonts should have segregated into two groups with a twofold difference in daily growth rates. But this was not the case. No evidence of segregation into groups was found; most of the F_1 haplonts were intermediate between the two original haploid parents. The essential data are given in table 5. The rate and amount of growth of these F_1 haplonts was normal in the presence of added thiamine.

The third experiment was made with the F_1 haplonts derived from the diplont 3 × 7. Both of the haploid parents made good growth in an essentially thiamine-free medium (table 3). The essential data are presented in table 6. The F_1 haplonts of the cross 3 × 7 more nearly resembled the original haploid parents than did the F_1 haplonts derived from the other two crosses. Only one of the F_1 haplonts, (3 × 7) D, failed to attain maximum weight in thiamine-free medium by the end of the experiment. The extreme variation in rate of growth among these isolates was two and nine-tenths fold. In the presence of added thiamine the growth of the F_1 haplonts was about equal to that attained by other haplonts. The average maximum weight was 132 milligrams.

HIGHLY PIGMENTED ISOLATES

A few of the single-spore isolates became deeply pigmented after the first few transfers on malt agar. In a few instances colored sectors developed in cultures which had been stable for months. The mycelium was dark brown in contrast to the light tan or straw color of the other isolates. A red-brown pigment diffused out of the mycelium. The production of this pigment appears to be correlated with death of the mycelium and subsequent lysis of the cells. The following observation supports this hypothesis. In liquid culture the medium of "normal" isolates seldom produced much color until about the time maximum weight was attained. After autolysis the culture liquid darkened rapidly. No chemical characterization of the pigment was attempted, but it was noted that the pigment behaved as an indicator. The color change was one of intensity rather than hue; the approximate pH range involved was 4 to 6.

All of these highly pigmented cultures were characterized by a slow rate of radial growth on agar medium. The transition to the colored state appeared to develop slowly in some instances; where colored sectors arose, the change was abrupt. The behavior of these colored cultures in liquid medium was also characteristic. The rate of growth in the presence of thiamine was rapid; the maximum weight was attained within 15 days for all colored cultures studied, and in one instance maximum weight was attained after 8 days of incubation. In general, the rate of growth in liquid medium without added thiamine was low. These experiments were continued for 46 days only. Although the maximum weight was attained by only two of the five cultures studied, the data indicate that the ability of these colored cultures to grow in the absence of added thiamine was variable. The data are given in table 7.

TABLE 7.—*Growth rates of colored cultures of Lenzites trabea in the absence and presence of added thiamine*

Isolate No.	No thiamine added			Thiamine added		
	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligram/days)	Length of incubation required to produce maximum weight of mycelium	Maximum weight of mycelium produced	Average daily increase in weight of mycelium (milligram/days)
	Days	*Milligrams*	*Milligrams*	*Days*	*Milligrams*	*Milligrams*
1	38	21	0.55	8	104	13.00
2	38	47	1.24	15	99	6.60
13	[1] 46	6	.13	15	89	5.93
7S [2]	[1] 46	16	.35	15	80	5.33
10S [2]	[1] 46	53	1.15	15	81	5.40

[1] Maximum weight not attained.
[2] Colored sectors.

Comparison shows that in the presence of thiamine the average maximum weight of these colored cultures was less than that of the "normal" isolates—91 milligrams as compared with 126 milligrams. The average rate of growth of the colored cultures in the presence of thiamine was somewhat higher than in the normal series, the lower maximum weights being counterbalanced by the shorter period of

incubation. In the absence of added thiamine the average rate of growth was less, in general, for the colored than for the "normal" isolates.

A direct comparison can be made between isolate 7 and 7S (a colored sector from isolate 7) and between isolate 10 and 10S (a colored sector from isolate 10). In the presence of thiamine, isolate 7 produced 58 milligrams more of mycelium than 7S, while isolate 10 produced 53 milligrams more of mycelium than 10S. In the absence of thiamine, isolate 7 grew at a rate some 13 times as great as the colored sector from this isolate. However, the colored sector from isolate 10 grew at nearly the same rate as the "normal" isolate. It is probable, therefore, that the highly pigmented state is not necessarily connected with the ability to synthesize thiamine.

The mycelium of these highly pigmented cultures was abnormal in that most of the cells were dead by the time maximum weight was attained. Microscopic examination showed that these dead cells were void of protoplasm. Most of the living cells were found at the hyphal tips.

The colored state appears to be irreversible. Although these observations were continued for more than a year, none of the colored cultures were ever observed to give rise to light-colored sectors.

Another abnormality of the colored cultures was observed in connection with fruiting. Most of the light-colored isolates (haploid and diploid) fruited readily (*2*), but fruiting was never observed in the colored cultures. Moreover, the colored cultures were no longer compatible with haplonts of the other group. Haplont 1, for example, when first isolated, was readily compatible with haplonts of the other compatibility group, but it lost this ability as soon as it changed to the pigmented form. The same was true of all the colored cultures.

In view of these observations, it was concluded that the highly pigmented or colored condition represents degenerative changes, the most fundamental of which is the loss of ability to pair and fruit. In nature such mutants would have little chance of survival.

DISCUSSION

This work illustrates the use of a higher basidiomycete for genetic studies. Some of the theoretical advantages to be expected from the use of this basidiomycete (*Lenzites trabea*) were not realized because of the intrinsic variability and slow rate of growth of this fungus. However, the diploid phase was available for cultural study under the same conditions as the haploid phase.

It is believed that the variability of this fungus is characteristic only of the species and not of the class as a whole. Others of the higher Basidiomycetes studied under identical conditions in this laboratory have yielded highly reproducible results.

Even in the presence of added thiamine the variability of this fungus was great. Especially was this true with respect to the time of incubation required to produce the maximum weight of mycelium. This intrinsic difference in rate of growth could not be correlated with degree of partial thiamine deficiency. It was impossible to predict the degree of partial thiamine deficiency from the growth rate in the presence of added thiamine.

That such wide variation exists among single-spore isolates from a single fruit body emphasizes again the danger of depending upon one or two single-spore isolates to characterize the biochemical nature of a fungus. Certainly in this instance the conclusions regarding partial thiamine deficiency of *Lenzites trabea* would have been vastly different had the experiments been carried on with isolate 18 or 30 only. Other instances of wide variation in biochemical character could be cited, as, for example, those found by Andes (*1*) in his study of the growth factor requirements of four monosporic lines of *Glomerella cingulata*.

That the degree of partial thiamine deficiency is not controlled by a single gene was the tenative conclusion reached by consideration of the data in table 1. Since these isolates were derived from the original fruit body, nothing was known concerning the partial thiamine deficiencies of the original haploid parents. Three experiments were made involving the use of haploid parents of known degree of partial thiamine deficiency. The results of these experiments (tables 4, 5, and 6) fully confirmed the above conclusion. Briefly, the degree of partial thiamine deficiency is inherited, but the mode of inheritance appears to be complex.

SUMMARY

Fourteen single-spore isolates of *Lenzites trabea* obtained from a single fruit body collected in nature showed considerable variability in rate of daily growth, ranging from 0.59 to 4.60 milligrams when cultured in a thiamine-free medium. These differences in growth in the absence of added thiamine were ascribed to differences in the capacity of the isolates to synthesize thiamine. In the presence of added thiamine all 14 isolates made nearly the same amount of growth but varied in rate of growth. Single-spore isolates from fruit bodies produced in culture from diplonts of known parent haplonts likewise varied widely in degree of partial thiamine deficiency. The amount of variation among these haplonts was far greater than could be ascribed to the normal variation found when the same isolates were repeatedly tested for partial thiamine deficiency. The variation in partial thiamine deficiency among these single-spore isolates must, therefore, be attributed to genetic origin.

The mode of inheritance of partial thiamine deficiency in *Lenzites trabea* appears to be complex, but at present we have no way of determining how many genes are involved.

Diplonts, derived from parent haplonts of known partial thiamine deficiency, were studied with respect to partial thiamine deficiency. In general, the following results were obtained: The diplonts exhibited far less variation in partial thiamine deficiency than did the haplonts; the degree of partial thiamine deficiency of the parent haplonts allowed no prediction as to the partial thiamine deficiency of the resulting diplont; the interaction of one nucleus upon another within a single cell appeared to be complex.

Single-spore isolates from haploid fruit bodies had similar, but not identical partial thiamine deficiencies. However, all such isolates resembled the parent haplont.

Colored sectors were produced by some haploid cultures. These aberrant forms were no longer capable of fruiting or pairing with isolates of the other compatibility group; such changes appeared to be irreversible.

LITERATURE CITED

(1) ANDES, J. O.
1946. EFFECT OF GROWTH SUBSTANCES ON GLOMERELLA CINGULATA. (Abstract) Phytopathology 36: 394.

(2) BARNETT, H. L., and LILLY, V. G.
1948. THE PRODUCTION OF HAPLOID AND DIPLOID FRUIT BODIES OF LENZITES TRABEA IN CULTURE. W. Va. Acad. Sci., Proc. (1947), *in* W. Va. Univ. Bul., Ser. 49, III–4: 34–39.

(3) BEADLE, G. W.
1945. GENETICS AND METABOLISM IN NEUROSPORA. Physiol. Revs. 25: 643–663, illus.

(4) BONNER, D.
1946. PRODUCTION OF BIOCHEMICAL MUTATIONS IN PENICILLIUM. Amer. Jour. Bot. 33: 788–791.

(5) BULLER, A. H. R.
1941. THE DIPLOID CELL AND THE DIPLOIDSATION PROCESS IN PLANTS AND ANIMALS, WITH SPECIAL REFERENCE TO THE HIGHER FUNGI. Bot. Rev. 7 (7–8): 335–431.

(6) FRIES, N.
1938. ÜBER DIE BEDEUTUNG VON WUCHSSTOFFEN FÜR DAS WACHSTUM VERSCHIEDENER PILZE. Symb. Bot. Upsal. 3 (2), 189 pp., illus.

(7) LEONIAN, L. H., and LILLY, V. G.
1945. THE COMPARATIVE VALUE OF DIFFERENT TEST ORGANISMS IN THE MICROBIOLOGICAL ASSAY OF B VITAMINS. W. Va. Agr. Expt. Sta. Bul. 319, 35 pp., illus.

(8) LILLY, V. G., and BARNETT, H. L.
1948. GROWTH RATES AND VITAMIN DEFICIENCIES OF VARIOUS FUNGI. W. Va. Acad. Sci., Proc. (1947), *in* W. Va. Univ. Bul., Ser. 49, III–4: 27–33.

(9) LINDEGREN, C. C.
1945. MENDELIAN AND CYTOPLASMIC INHERITANCE IN YEASTS. Mo. Bot. Gard. Ann 32: 107–123, illus.

(10) MOUNCE, I., and MACRAE, R.
1936. THE BEHAVIOR OF PAIRED MONOSPOROUS MYCELIA OF LENZITES SAEPIARIA (WULF.) FR., L. TRABEA (PERS.) FR. L. THERMOPHILA FALCK, AND TRAMETES AMERICANA OVERH. Canad. Jour. Res. 14: [215]–221, illus.

(11) ROBBINS, W. J., and MA, R.
1942. VITAMIN DEFICIENCIES OF CERATOSTOMELLA. Torrey Bot. Club Bul. 69: 184–203, illus.

A POD-DISTORTING STRAIN OF THE YELLOW MOSAIC VIRUS OF BEAN[1]

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INTRODUCTION

During the summer of 1946 in an experimental plot near Madison, Wis., plants of Idaho Refugee and other varieties of bean (*Phaseolus vulgaris* L.) highly resistant to bean virus 1 appeared to be affected with yellow mosaic (bean virus 2), but were more severely stunted than typical and bore severely distorted pods. Preliminary inoculations to bean in the greenhouse indicated that the disease was incited by a virus and that plants of certain varieties developed severe top necrosis while others were highly resistant. In parallel inoculation tests with the typical strain of bean virus 2, mottle and slight stunting occurred, but no varieties were highly resistant and in only one variety did the top necrosis develop. The inoculations indicated that this virus was not the typical strain of bean virus 2, but similarity of symptoms on certain bean varieties suggested that it might be a strain of that virus. This paper is a report of the results of a comparative study of this apparently new virus strain with other viruses of bean.

MATERIALS AND METHODS

The greenhouses in which studies were conducted were kept free of insects by frequent fumigation and spraying. All inoculations and other tests were made in greenhouses in which the temperature was maintained between 24° and 28° C.

A culture of typical bean virus 2 was secured from naturally infected plants in the vicinity of Madison, Wis. This virus is the same as that used in studies reported recently (*2*, *3*),[3] and it is believed to be similar to the strain described by Pierce (*7*). A culture of the pod-distorting virus was obtained from naturally infected Idaho Refugee plants also in the vicinity of Madison. Stock cultures of these viruses were maintained in Sensation Refugee 1066 plants, which variety is highly resistant to bean virus 1. The possibility of contamination with the latter virus was thereby practically eliminated. Sources of several other viruses and virus strains used in cross-protection studies will be given later.

[1] Received for publication January 26, 1948.
[2] The writers are indebted to Eugene H. Herrling for preparation of the illustrations.
[3] Italic numbers in parentheses refer to Literature Cited, p. 314.

Lot Z–1 and the variety Rival were supplied by W. J. Zaumeyer. The UI selections were obtained from Leslie Dean of the University of Idaho. The other bean varieties used were obtained from reliable commercial sources. Seeds of the various legumes used were secured from Roland McKee of the United States Department of Agriculture.

All greenhouse plants were inoculated in early stages of growth by rubbing with carborundum as an abrasive. At least 10 plants were used for each inoculation test. Where symptoms on inoculated plants were mild or completely absent, the presence or absence of the viruses was determined by extracting the juice and inoculating healthy Idaho Refugee plants.

The properties of the pod-distorting virus were determined on greenhouse-grown plants by the methods described by Johnson and Grant (*4*).

EXPERIMENTAL RESULTS

INOCULATIONS TO BEAN VARIETIES

Symptom reactions of 37 varieties or strains of bean to the pod-distorting virus and the common strain of bean virus 2 are presented in table 1. They can be grouped with respect to the pod-distorting virus into four classes: (1) No symptoms and no recovery of the virus; (2) stunt, mottle, and leaf deformation but no necrosis (fig. 1); (3) top necrosis followed by partial recovery or general necrosis followed by death of the entire plant (fig. 2 and 3); (4) a modified

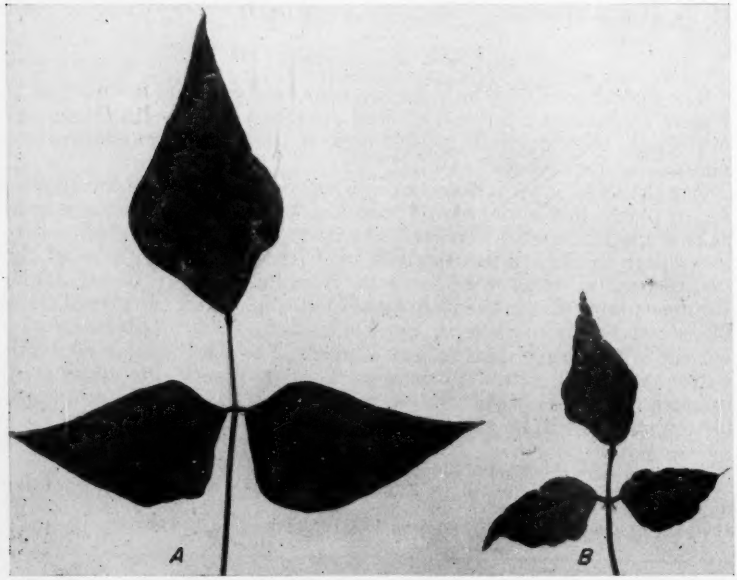

FIGURE 1.—Symptoms on leaves of Idaho Refugee bean plants infected with typical yellow mosaic virus (*A*) and the pod-distorting virus (*B*).

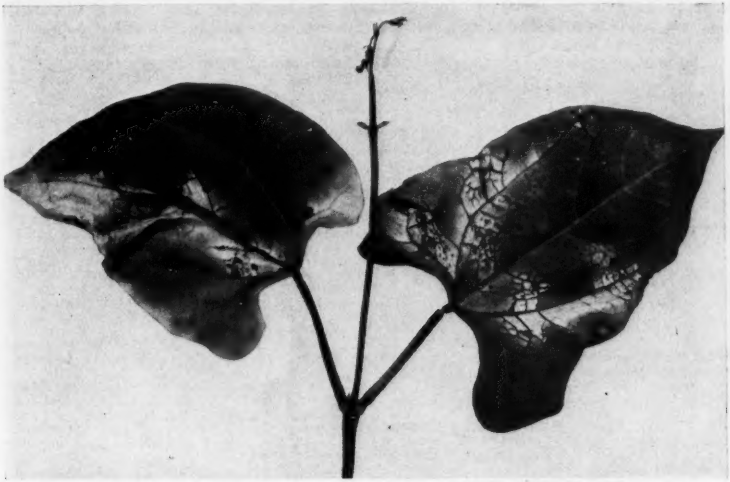

FIGURE 2.—Necrosis on inoculated leaves and on the growing point of a bean plant of the UI 15 variety.

type of necrotic reaction in which the tip leaves turned yellow and were abscised leaving a bare stem at the extremities but not killing the growing point (fig. 4).

Pods which were set on plants of any group with mottle or other symptoms were severely warted and misshapen (fig. 5). The typical strain of yellow bean mosaic virus was infectious to all varieties of bean tested, usually causing a stunt and mottle but no necrosis. However, it caused a necrotic reddening of stem and petiole at the nodes of the plants of Stringless Blue Lake (fig. 6) which resembled the disease described by Virgin (*9*). On McCaslan it caused top necrosis which resembled the symptoms produced by the pod-distorting virus on Michelite (fig. 7), Pinto, and several other varieties listed in table 1. However, in no case did it produce malformed and warted pods. The varieties UI 59, UI 81, and UI 123 were highly resistant to the pod-distorting virus and attempts to recover the virus from them were unsuccessful.

RANGE OF LEGUMINOUS HOSTS

Nineteen leguminous hosts listed in table 2 were inoculated with typical bean virus 2 and the pod-distorting virus to determine whether the latter virus would infect the same hosts as the former, and, if so, whether any differences existed between the two in symptom expression. A study of table 2 will reveal that 7 of the hosts were infected by both of the viruses, symptoms appearing either as a mottle or a vein-clearing mottle. Symptoms incited by typical bean virus 2 and the pod-distorting virus on Canadian field pea, fenugreek, and crimson clover are shown in figure 8. None of the other 12 hosts were infected by the pod-distorting virus but 6 of them were infected by typical bean virus 2. The pod-distorting virus did not infect any host which was

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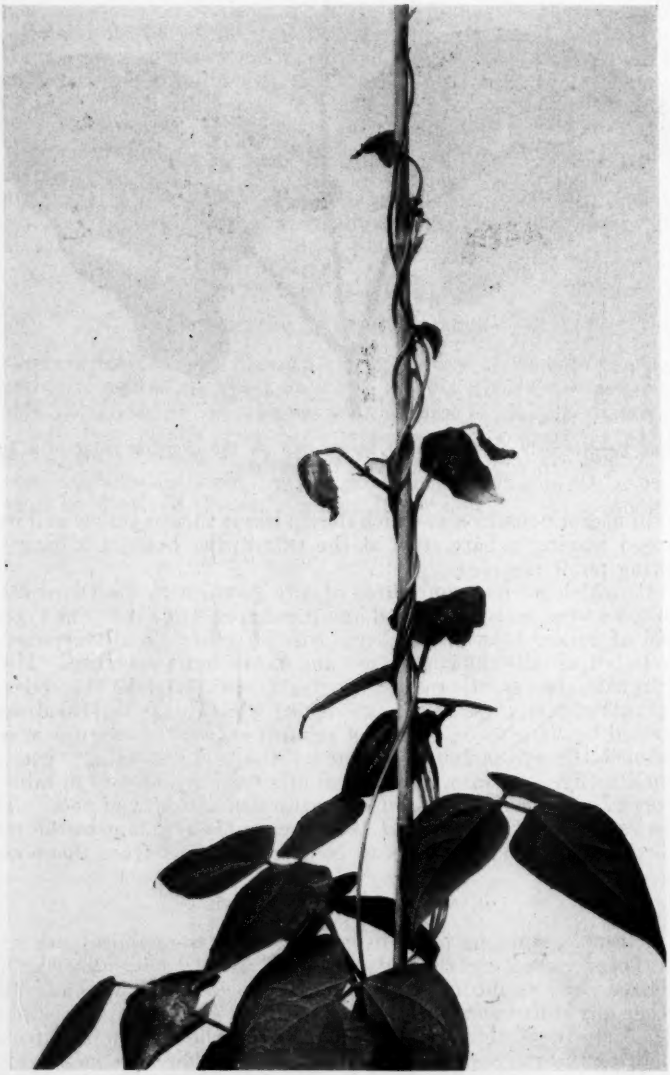

FIGURE 3.—Top necrosis on a plant of the UI 1 variety of bean infected with the pod-distorting virus.

TABLE 1.—*Symptoms produced on plants of 37 varieties or strains of bean inoculated with typical bean virus 2 and on plants inoculated with the pod-distorting virus*

Variety or strain	Symptoms on plants inoculated with—	
	Typical bean virus 2	Pod-distorting virus
UI 59	Faint mottle	None.
UI 81	do	Do.
UI 123	do	Do.
Michelite	Distinct mottle, slight stunt	Top necrosis.
UI 1	do	Do.
UI 15	do	Do.
Pinto	do	Do.
Stringless Blue Lake (black-seeded)	Distinct mottle, slight stunt, and red nodes.	General necrosis of old plants.
Stringless Blue Lake (white-seeded)	do	Top necrosis.
Sensation Green Pod	Distinct mottle and moderate stunt.	Do.
Bountiful	do	Do.
Potomac	do	Necrosis of main stem of old plants but no top necrosis.
Kentucky Wonder	do	Do.
Keystonian	do	Top necrosis.
Golden Cluster Wax	do	Do.
Scotia or Striped Creaseback	Severe stunt, mottle	All plants dead.
Lazy Wife	do	Top necrosis.
McCaslan	Mottle and top necrosis	Do.
Resistant Kentucky Wonder	Moderate stunt, mottle	Necrosis of main stem in old plants.
Robust	do	Mild top necrosis.
New Stringless	do	Leaves twisted and deformed; young leaves abscised.
Full Measure	do	Do.
Pencil Pod Black Wax	do	Do.
Tenderpod	do	Do.
Stringless Black Valentine	do	Do.
Burpee Brittle Wax	do	Do.
Tendergreen	do	Do.
Rival	do	Do.
Giant Stringless Green Pod	do	Severe stunt, mottle, and leaf distortion.
Dwarf Horticultural	do	Moderate stunt, deformed leaves, mottle.
Rust Proof Golden Wax	do	Do.
Plentiful	do	Do.
Improved Golden Wax	do	Do.
Florida Belle	do	Do.
Z-1	do	Do.
Sensation Refugee 1066	do	Do.
Idaho Refugee	do	Do.

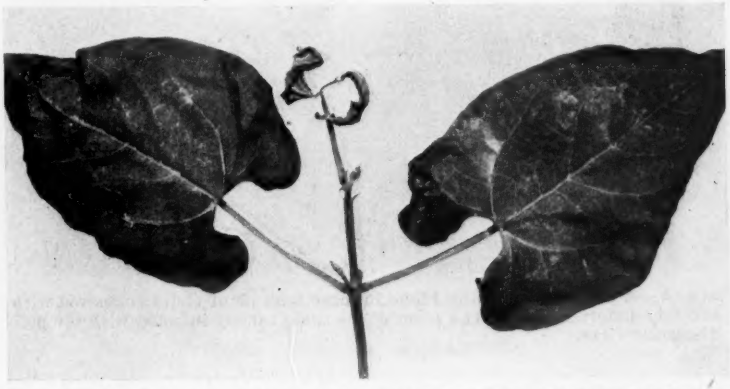

FIGURE 4.—A plant of the Rival variety of bean infected with the pod-distorting virus; one leaflet of the youngest trifoliate leaf has dropped, the other two are dead, but the growing point is not necrotic.

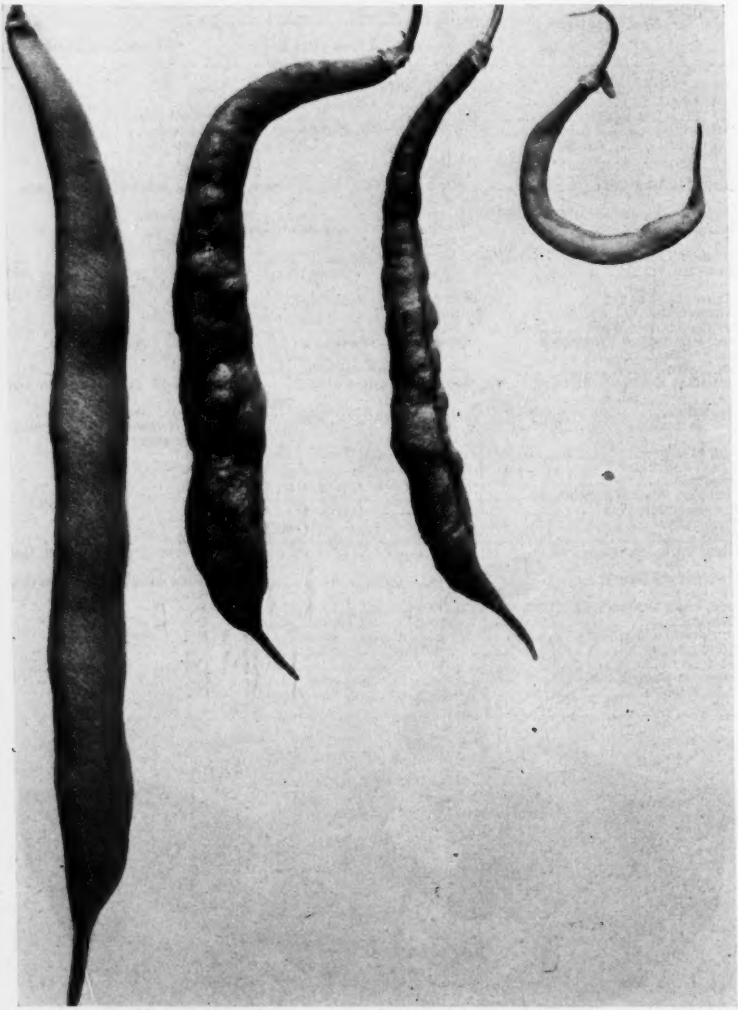

FIGURE 5.—A pod from a healthy Idaho Refugee bean plant (left) compared with severely distorted pods from a plant of the same variety infected with the pod-distorting virus.

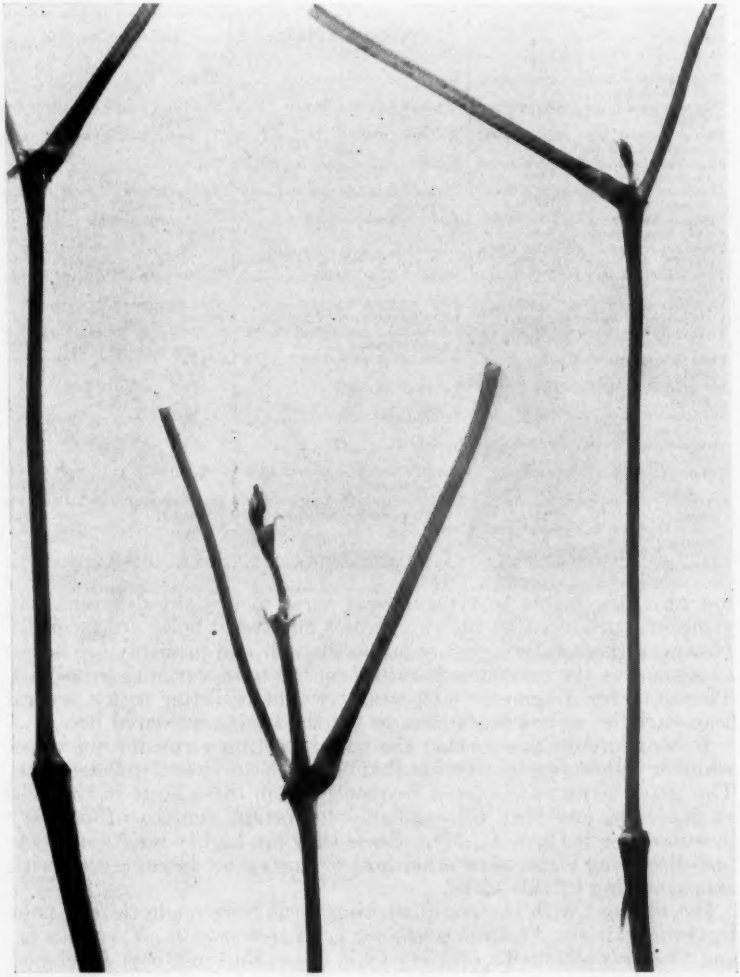

FIGURE 6.—Necrosis at the juncture of stem and petiole in a plant of the Stringless Blue Lake variety of bean infected with typical bean virus 2.

TABLE 2.—*Symptoms produced by typical bean virus 2 and the pod-distorting virus on 18 leguminous hosts other than bean*

Host	Symptoms produced by—	
	Typical bean virus 2	Pod-distorting virus
Pisum sativum L. (pea var. Wisconsin Perfecton).	None	None.
Pisum sativum L. (pea var. Perfected Wales).	Distinct yellowish mottle	Yellowish mottle and slight vein clearing.
Pisum sativum var. *arvense* Poir. (Canadian field pea).	Yellowish mottle	Yellowish mottle and vein clearing.
Phaseolus lunatus L. (Henderson Bush lima).	None	None.
Trifolium incarnation L. (crimson clover).	Reduction in leaf size and vein clearing.	Yellowish-green mottle with vein clearing.
Trifolium hybridum L. (alsike clover).	Faint yellowish mottle	Faint green mottle.
Trifolium pratense L. (red clover)	None	None.
Trifolium repens L. (Ladino white clover).	do	Do.
Vicia monantha (L.) Desf. (Monantha vetch).	Yellow-spotted mottle	Yellow to white-spotted mottle.
Vicia villosa Roth. (hairy vetch)	Faint greenish mottle	Very faint greenish mottle.
Vicia faba L. (Broadbean)	Light greenish-yellow mottle	None.
Vicia sativa L. (spring vetch)	Leaves light green with darker green islands.	Do.
Vicia atropurpurea Desf. (purple vetch).	Yellowish-green mottle	Do.
Melilotus alba Desr. (white sweetclover).	Yellowish mottle	Do.
Melilotus officinalis (L.) Lam. (yellow sweetclover).	Yellow-green mottle	Do.
Medicago hispida Gaertn. (Bur clover).	None	Do.
Soja max (L.) Piper (soybean)	Crinkled upper leaves with yellow mottle.	Do.
Trigonella foenum-graecum L. (fenugreek).	Leaves yellow green with darker green islands.	Yellowish mottle and slight vein clearing.
Arachis hypogaea L. (peanut var. Jumbo).	None	None.

not also susceptible to typical bean virus 2. Slight differences in symptoms produced by the two viruses on several hosts are recorded. However, these differences are not as distinct and probably are not as consistent as the reactions described on the bean varieties in table 1. Therefore for diagnostic purposes, it would be better to use several bean varieties as test hosts than to use the hosts considered here.

It is of interest to note that the pod-distorting virus did not infect white or yellow sweetclover, but that typical bean virus 2 infected both. The latter virus was isolated frequently from these hosts in the field at Madison, and they are probably important sources of primary inoculum for it (*7*, *8*, *14*, *15*). Since they are highly resistant to the pod-distorting virus, some other host or hosts must be concerned with overwintering of this virus.

Inoculations with the pod-distorting virus were made to four nonleguminous hosts, *Nicotina glutinosa* L., *N. tabacum* L., *N. rustica* L., and *Cucumis sativus* L. (variety Ohio 31). No symptoms developed and attempts to recover the virus by inoculating bean failed in all cases.

PROPERTIES OF THE POD-DISTORTING VIRUS

Expressed juice from Idaho Refugee plants recently infected with the virus was treated and then used to inoculate young healthy Idaho Refugee plants. Five plants were used as a test unit and each experiment was repeated three times. The properties of the pod-distorting virus as shown in table 3 were as follows: Thermal inactivation point,

after heating for 10 minutes, 58° to 60° C.; inactivation in vitro at 20°, 32 hours; dilution end point, 1–2,000. These properties conform to those reported by Pierce (*7*) for typical bean virus 1 and typical bean virus 2.

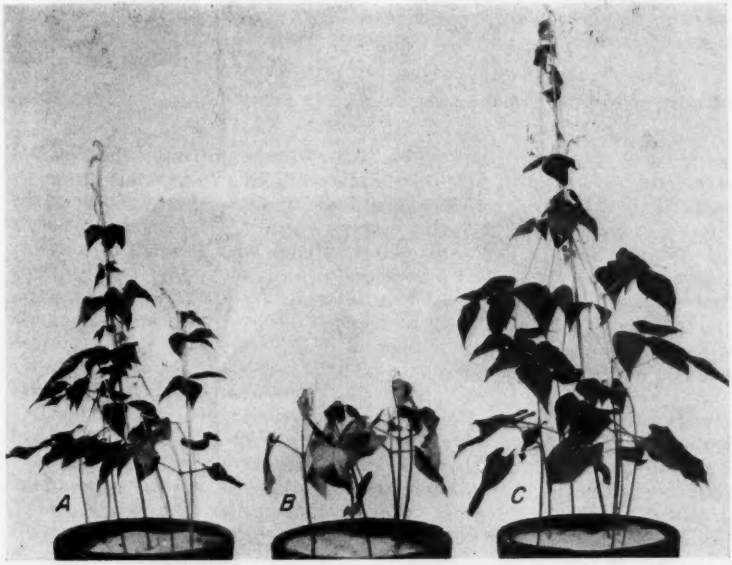

FIGURE 7.—*A*, Plants of the Michelite variety of bean infected with typical bean virus 2; stunting and mottle occur but there is no necrosis. *B*, Plants of the same variety infected with the pod-distorting virus and severely affected with top necrosis. *C*, Uninoculated plants of Michelite variety.

TABLE 3.—*Properties of the pod-distorting virus*

Thermal inactivation			Tolerance to dilution			Longevity in vitro		
Temperature (°C.)	Total plants	Infected plants	Dilution	Total plants	Infected plants	Hours	Total plants	Infected plants
	Number	*Number*		*Number*	*Number*		*Number*	*Number*
Untreated	15	15	Undiluted	15	15	0	15	15
45	15	14	1–10	15	15	12	15	15
50	15	15	1–100	15	15	24	15	3
52	15	15	1–1,000	15	7	32	15	0
54	15	11	1–1,200	15	6	48	15	0
56	15	6	1–1,400	15	3	60	15	0
58	15	3	1–1,600	15	4			
60	15	0	1–1,800	15	1			
65	15	0	1–2,000	15	0			
70	15	0	1–2,200	15	0			
80	15	0	1–10,000	15	0			

SEED TRANSMISSION

Pierce (*7*) reported no infection in 6,532 seedlings grown from seed of plants infected with bean virus 2, while seed transmission of bean virus 1 was readily demonstrated. When 285 seeds from Idaho Refugee plants artificially infected with the pod-distorting virus in the

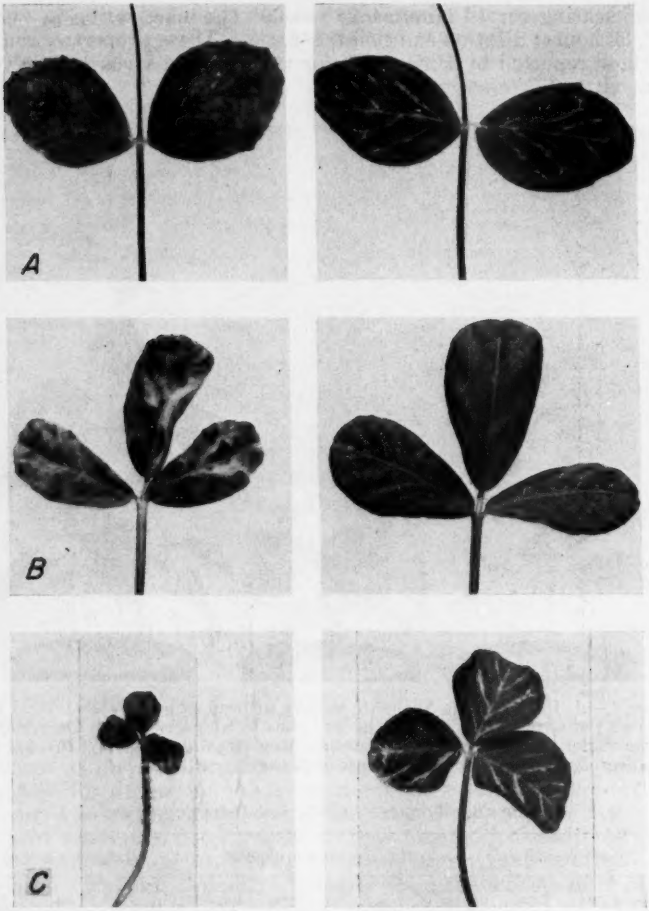

FIGURE 8.—Leaves from bean plants infected with typical bean virus 2 (left) and the pod-distorting virus (right): *A*, Canadian field pea; *B*, fenugreek; *C*, crimson clover.

greenhouse were planted in flats in the greenhouse, no disease occurred. When 160 seeds from naturally infected Sensation Refugee 1066 plants infected in the field were tested in the same manner, no plants developed disease. Although further trials are needed to determine conclusively whether this virus is ever transmitted through bean seeds, these preliminary tests indicate strongly that it is not.

INSECT TRANSMISSION

The typical strain of bean virus 2 is transmitted by several species of aphids (*7*). Two trials were conducted to determine whether the pod-distorting virus is insect-transmitted. Green peach aphids

(*Myzus persicae* (Sulz.)) were raised on healthy cabbage plants, removed to a clean, dry test tube, and starved for 3 hours. After this interval they were allowed to feed on plants infected with the pod-distorting virus for 15 to 20 minutes. Five or more aphids were then transferred to each healthy test plant and allowed to feed until the insects died. In one test two plants out of six inoculated became infected with the pod-distorting virus; in a second test three plants out of six inoculated were infected.

CROSS PROTECTION BY SEVERAL VIRUSES OF BEAN AGAINST THE POD-DISTORTING VIRUS

The pod-distorting virus caused severe top necrosis on several varities of bean, two of which were Michelite and Pinto. This distinctive reaction provided a test to determine whether or not several other viruses of bean which cause systemic infection but no necrosis could prevent or decrease the incidence of necrosis caused by the pod-distorting virus if they were applied to the test plants several days in advance of the latter. When strains of bean virus 2 and Burkholder's strain of bean virus 1 were being tested, plants of the Michelite variety were used for the test. When the typical strain of bean virus 1 was used it was necessary to use Pinto plants since Michelite is not susceptible to that strain.

The viruses used were typical bean virus 2, greasy-pod virus, Burkholder's strain of bean virus 1, and typical bean virus 1 from stock cultures described previously (*2*, *3*). In addition to these, there was included a culture of southern bean mosaic (*11*, *12*) obtained from W. C. Price and a culture of cucumber virus 1 (strain 14), which is infectious to bean and pea (*10*). This latter virus was obtained from a stock culture maintained in this laboratory. These viruses were inoculated to the test plants 15 days in advance of the pod-distorting virus and final readings on necrosis produced were made 1 month after the second inoculation, a period sufficient to permit the maximum development of necrosis. Results are presented in table 4.

TABLE 4.—*Cross protection by 5 viruses of bean against the pod-distorting virus*

Treatment	Test variety	Total plants	Necrotic plants
Typical bean virus 2 followed by the pod-distorting virus [1]	Michelite	41	3
Cucumber virus 1 (strain 14) followed by the pod-distorting virus	do	18	18
Greasy-pod virus followed by the pod-distorting virus	do	42	38
Southern bean mosaic virus followed by the pod-distorting virus	do	17	14
Pod-distorting virus only	do	40	38
Greasy pod virus followed by the pod-distorting virus	Pinto	30	2
Typical bean virus 1 followed by the pod-distorting virus	do	30	1
Pod-distorting virus only	do	30	29

[1] All second inoculations were made 15 days after the first.

When susceptible plants were inoculated with typical bean virus 2, greasy-pod virus, typical bean virus 1, or Burkholder's strain of bean virus 1, 15 days before inoculation with the pod-distorting virus, there was a marked reduction in the amount of top necrosis produced by the pod-distorting virus. However, similar inoculations with southern bean mosaic virus or cucumber virus 1 (strain 14) caused no reduction in top necrosis. When Michelite plants were inoculated

with the greasy-pod virus and later inoculated with the pod-distorting virus, no reduction in the amount of top necrosis resulted. In contrast to this, when similar inoculations were made to plants of the Pinto variety a marked reduction in the amount of top necrosis occurred. This is explained by the fact that Michelite is highly resistant to the greasy-pod virus while Pinto is susceptible. Therefore when Michelite was inoculated with the greasy-pod virus no infection resulted and the subsequent inoculation with the pod-distorting virus was made to healthy plants which developed top necrosis to as great an extent as the control plants (pod-distorting virus alone).

In order to determine whether or not the suppression of the top-necrosis symptom described above was due to a complete or nearly complete inhibition of development of the pod-distorting virus, the following experiment was carried out. Fifteen Stringless Green Refugee plants (susceptible to both bean virus 1 and the pod-distorting virus) were inoculated with bean virus 1 at the two-leaf stage and 15 days later 10 of these plants were inoculated with the pod-distorting virus. Five plants were thereby left with only the bean virus 1 inoculation. At this same time five healthy plants of the same age were inoculated with the pod-distorting virus and five plants were left uninoculated to serve as controls. Observations made 20 days later indicated that the pod-distorting virus was not present in the plants which were infected with bean virus 1 at the time of inoculation with the former virus, since the plants thus treated could not be distinguished from those which had been inoculated with only bean virus 1. However, those healthy plants which had been inoculated with the pod-distorting virus at the time of the second inoculation were severely diseased. In order to determine whether the pod-distorting virus was entirely absent, or present and masked, a composite inoculum was made by taking a small trifoliate leaf from each plant, grinding them together, and inoculating five plants each of Michelite and Stringless Green Refugee. These inoculations indicated that bean virus 1 had completely inhibited the development of the pod-distorting virus since the Michelite plants remained symptomless while the Stringless Green Refugee plants were severely affected with common mosaic.

COMPARISON WITH OTHER STRAINS OF BEAN VIRUS 2

Several strains of bean virus 2 have been described. Virgin (*9*) described a disease which occurs on bean in some of the Western States the incitant of which he believed to be a strain of bean virus 2. A culture of this virus was received from Virgin and inoculations to several varieties of bean indicated that it was identical with or at least very similar to the virus referred to in this paper as typical bean virus 2. Both of these strains caused the necrotic joints or "red node" symptoms described by Virgin (*9*). McWhorter et al. (*6*) described a severe virus disease of bean in Oregon which usually occurred in fields near plantings of gladiolus, which were thought to be the source of inoculum in nature. Two isolates were furnished by McWhorter under numbers 231 and 250. Isolate 231 caused death of pea plants (Prince of Wales variety) but caused no necrosis in any of the bean varieties inoculated. It failed to infect any of the Refugee varieties

(Idaho Refugee, Stringless Green Refugee, Sensation Refugee 1066, and Sensation Refugee 1071) tested and is therefore different from any of the viruses considered in this paper. Isolate 250 was not recovered from the infected plant tissue received. LeBeau (*5*) reported a virus-induced top necrosis of bean in Mississippi. However, his virus infected tobacco and cucumber and had a heat inactivation point of 68° C. It is therefore different from the pod-distorting virus described in this paper.

DISCUSSION

On the basis of symptoms, modes of transmission, physical properties, and cross-protection studies the pod-distorting virus found on bean in Wisconsin appears to be closely related to typical bean virus 2 as described by Pierce (*7*). It has been shown in a previous paper (*3*) that bean virus 1 is closely related to bean virus 2. Cross-protection studies presented in this paper indicate that the pod-distorting virus is also related to bean virus 1. Since the former virus is not transmitted in bean seed, however, it is probably more closely related to bean virus 2 than to bean virus 1. The reactions of the bean varieties inoculated with typical bean virus 2 and pod-distorting virus also show a close relationship between the two viruses. Varieties UI 59, UI 81, and UI 123 which were most tolerant to the typical strain, were highly resistant to the pod-distorting virus. Varieties Stringless Blue Lake, Striped Creaseback, and McCaslan, which were the most susceptible to the typical strain, developed severe necrosis, and were sometimes killed when affected with the pod-distorting virus. Yellow bean mosaic is a very important disease in western canning areas where Stringless Blue Lake is the variety used most commonly. It is not known whether the pod-distorting strain occurs in these areas, but if it should become prevalent it would probably cause as much or more damage than has been reported for the typical strain.

Since the white and yellow sweetclovers are immune or highly resistant to the pod-distorting virus they cannot serve as overwintering hosts as they do commonly for typical bean virus 2. Further study is needed to determine the hosts in which the pod-distorting virus overwinters.

Several other viruses which might be strains of bean virus 2 were studied to determine their similarity to or difference from the pod-distorting virus. All of these viruses were shown to differ in one or more ways and are therefore distinct from the virus considered here. More work is needed to characterize them.

SUMMARY

A pod-distorting virus isolated from naturally infected bean plants at Madison, Wis., was studied in comparison with the typical strains of bean virus 1 and bean virus 2 and several other bean viruses. Evidence is presented to show that this virus is closely related to bean virus 1 and bean virus 2. Because of symptoms on certain hosts and because it is probably not seed-transmitted, it is thought to be a strain of bean virus 2.

The typical strain of bean virus 2 infected all varieties of bean tested, while the pod-distorting virus infected all but UI 59, UI 81, and

UI 123. The latter virus caused severe top necrosis on several varieties which when infected with typical bean virus 2 developed only a slight stunt and mottle.

Of 20 other leguminous varieties and species tested, 7 were susceptible to both viruses, 6 were susceptible to typical bean virus 2 and highly resistant to the pod-distorting virus, and 7 were not infected by either virus.

LITERATURE CITED

(1) BLOOD, H. L.
1947. AN UNUSUALLY HEAVY INFECTION OF YELLOW BEAN MOSAIC DESTROYS THE CACHE VALLEY BEAN CROP. U. S. Bur. Plant Indus., Plant Dis. Rptr. 31: 384. [Processed.]

(2) GROGAN, R. G., and WALKER, J. C.
1948. INTERRELATION OF BEAN VIRUS 1 AND BEAN VIRUS 2 AS SHOWN BY CROSS-PROTECTION TESTS. Phytopathology 38: 489–493.

(3) ——— and WALKER, J. C.
1948. THE RELACION OF COMMON BEAN MOSAIC TO BLACK ROOT OF BEAN. Jour. Agr. Res. 77: 315–331, illus.

(4) JOHNSON, J., and GRANT, T. J.
1932. THE PROPERTIES OF PLANT VIRUSES FROM DIFFERENT HOST SPECIES. Phytopathology 22: 741–757.

(5) LEBEAU, F. J.
1947. A VIRUS-INDUCED TOP NECROSIS IN BEAN. (Abstract) Phytopathology 37: 434.

(6) MCWHORTER, F. P., BOYLE, L., and DANA, B. F.
1947. PRODUCTION OF YELLOW BEAN MOSAIC IN BEANS BY VIRUS FROM MOTTLED GLADIOLUS. Science 105: 177–178.

(7) PIERCE, W. H.
1934. VIRUSES OF THE BEAN. Phytopathology 24: 87–115, illus.

(8) ———.
1935. THE IDENTIFICATION OF CERTAIN VIRUSES AFFECTING LEGUMINOUS PLANTS. Jour. Agr. Res. 51: 1017–1039, illus.

(9) VIRGIN, W. J.
1943. AN UNUSUAL BEAN DISEASE. (Phytopath. note) Phytopathology 33: 743–745, illus.

(10) WHIPPLE, O. C., and WALKER, J. C.
1941. STRAINS OF CUCUMBER MOSAIC VIRUS PATHOGENIC ON BEAN AND PEA. Jour. Agr. Res. 62: 27–60, illus.

(11) ZAUMEYER, W. J., and HARTER, L. L.
1942. A NEW VIRUS DISEASE OF BEAN. (Phytopath. note) Phytopathology 32: 438–439.

(12) ——— and HARTER, L. L.
1943. TWO NEW VIRUS DISEASES OF BEANS. Jour. Agr. Res. 67: 305–328 illus.

(13) ——— and THOMAS, H. R.
1947. BEAN DISEASES IN SOME OF THE INTERMOUNTAIN STATES IN 1946. U. S. Bur. Plant Indus., Plant Dis. Rptr. 31: 59–65. [Processed.]

(14) ——— and WADE, B. L.
1935. THE RELATIONSHIP OF CERTAIN LEGUME MOSAICS TO BEAN. Jour. Agr. Res. 51: 715–749, illus.

(15) ——— and WADE, B. L.
1936. PEA MOSAIC AND ITS RELATION TO OTHER LEGUME MOSAIC VIRUSES. Jour. Agr. Res. 53: 161–185, illus.

THE RELATION OF COMMON MOSAIC TO BLACK ROOT OF BEAN[1]

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INTRODUCTION

There are now in common use several varieties of bean (*Phaseolus vulgaris* L.) which are highly resistant to bean virus 1, the incitant of common mosaic. The resistant property in certain of these has been derived from Corbett Refugee, which in turn was the progeny of a highly resistant plant that appeared in a field of infected Stringless Green Refugee (*9*, *10*, *11*).[3] In this group of resistant varieties are Idaho Refugee, Wisconsin Refugee, Sensation Refugee 1066, Sensation Refugee 1071, and U. S. No. 5 Refugee. Robust and Michelite are resistant varieties of different origin. Several resistant varieties have been developed by selection from Great Northern field bean at the Idaho Agricultural Experiment Station and some of these have been used as parents in crosses with other varieties.

All of the varieties mentioned, insofar as reported, are immune from bean virus 1 in that they do not become infected when inoculated by mechanical inoculation (rubbing method) and the virus is not recovered from the inoculated plants. They are susceptible to at least some of the strains of bean virus 2, the incitant of yellow mosaic. It has been shown by Pierce (*11*) that resistance to bean virus 1 of these groups, as represented by Idaho Refugee, is distinct in its genetic inheritance from that of the other two groups.

During the course of studies of several strains of bean virus 1 and bean virus 2 it was noted that an occasional plant of Idaho Refugee developed systemic necrosis and died. Repeated isolations failed to yield any bacterial or fungus pathogen and numerous inoculations with bean virus 2 failed to produce this necrotic symptom. Only when the plants were inoculated with bean virus 1 alone or with a virus mixture containing both viruses did the necrosis appear. It was of interest, therefore, to determine whether bean virus 1 was actually the cause of the systemic necrosis, and if so, whether other varieties that are resistant to this virus also give the same necrotic reaction.

In the earlier phases of this study inoculations were made only on young plants, and those which became infected died without producing

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[2] The writers are indebted to R. H. Larson for advice during the investigation and to Eugene H. Herrling for preparation of the illustrations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 331.

any pods. However, on one occasion inoculation was delayed until pods had formed and the necrosis appeared on the stems, pods, and roots, the symptoms conforming closely with those described by Jenkins for black root (*6*, *7*).

This investigation is concerned with the reactions of bean varieties to three strains of bean virus 1 and to the typical strain of bean virus 2 with special emphasis on the occurrence of systemic necrosis in young and in maturing plants.

MATERIALS AND METHODS

Stock cultures of the viruses were maintained in susceptible Stringless Green Refugee plants grown from virus-free seed in the greenhouse. The greenhouses in which studies were conducted were kept free of insects by frequent fumigation and spraying. The experimental plants were grown at temperatures usually ranging from 24° to 28° C. Several methods of inoculation were used and each will be described in its appropriate section under Experimental Results.

The common mosaic and greasy pod strains of bean virus 1 were obtained from infected seed and leaf material, respectively, supplied by W. J. Zaumeyer. Zaumeyer and Thomas (*19*) reported that symptoms of greasy pod differed from those of common mosaic, but gave no other means of distinguishing one from the other. Plants inoculated with either greasy pod or common mosaic showed leaf curling and mottling characteristic of common mosaic and occasionally greasiness of the pods on such susceptible varieties as Stringless Green Refugee and Pinto. These viruses were very difficult if not impossible to distinguish on the basis of symptoms and reacted similarly on all varieties under the conditions used in this investigation. Burkholder's (*2*) strain of bean virus 1 differs from the common strain in that it is infectious to Robust, Michelite, Great Northern UI 15, and several other varieties that are resistant to the common strain. A culture of this virus was obtained from plants grown from infected Michelite bean seed supplied by Dr. Burkholder, and the virus will be referred to throughout this paper as "Burkholder's strain." Bean virus 2 was secured from naturally infected plants in the vicinity of Madison, Wis.

Bean varieties in commercial use were secured from reliable commercial sources. Dr. Zaumeyer provided samples of several breeding lines, all of which are highly resistant to bean virus 1 and derive their resistance from the same group as Idaho Refugee. C18 and C19 were selections made at the Wisconsin Experiment Station, from a cross between Idaho Refugee and New Stringless, and carry the same type of resistance as the former parent.

EXPERIMENTAL RESULTS

MECHANICAL INOCULATION OF YOUNG RESISTANT PLANTS

Twenty young plants of the variety Sensation Refugee 1066 were inoculated with the common mosaic strain of bean virus 1, 20 with bean virus 2, and 20 with a mixture of the two viruses. The inoculum consisted of juice freshly extracted from infected plants applied to the primary leaves with carborundum as an abrasive.

Four plants inoculated with bean virus 1 showed systemic necrosis and eventually died; none of those inoculated with bean virus 2 became necrotic but all developed mottle and stunting typical of yellow mosaic. Five of the plants inoculated with a mixture of the viruses showed systemic necrosis and died; on the remaining plants symptoms of yellow mosaic developed. This experiment, which was repeated several times with similar results, showed that bean virus 1 was responsible for the necrosis and its development was not influenced by the presence of bean virus 2.

The first evidence of necrosis in Sensation Refugee 1066 plants was browning of the veinlets of inoculated leaves. The browning extended to the midvein and to the petiole and it appeared as brown or reddish-brown streaks beneath the epidermis of the stem. When the outer layer of the taproot was peeled back a reddish-brown area was found, and this area increased until the entire cortex showed brown to black discoloration. The next symptom was a browning of the veinlets and flecking of interveinal tissue of unfolding leaves, which died quickly, followed shortly by death of the entire plant. Plants have not been observed to recover from necrosis. A typical young plant affected with necrosis is shown in figure 1.

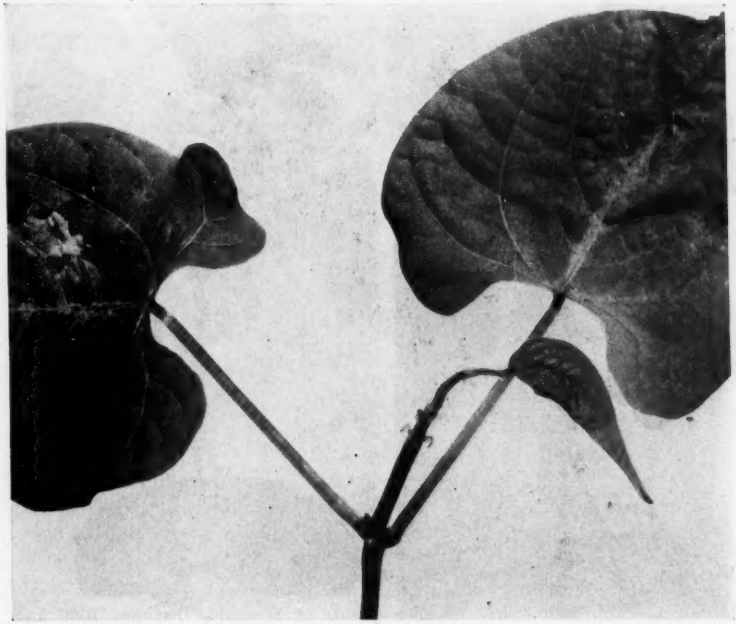

FIGURE 1.—A young plant of Sensation Refugee 1066 inoculated by rubbing the primary leaves with juice from a plant inoculated with bean virus 1. Stem necrosis is pronounced and flecks and darkening of veinlets appear in the youngest leaflet at the growing tip. Two of the trifoliate leaves have dropped.

When juice from plants in the early stage of necrosis was used as inoculum on Stringless Green Refugee plants, which are very susceptible to the common-mosaic strain of bean virus 1, typical common mosaic developed. When plants in the advanced stages of necrosis were used similarly as a source of inoculum no symptoms developed on plants of the susceptible variety. The virus evidently remained viable in the resistant host only during the early stages of disease development.

INOCULATION BY APPROACH GRAFTS

In view of the relatively low percentage of plants infected by mechanical inoculation, the approach-graft method of inoculation was tried. Seeds of the resistant variety, Sensation Refugee 1066, and of the susceptible variety, Stringless Green Refugee, were planted in 6-inch pots in separated rows. After the plants were in the two-leaf stage a shallow slice was taken from the stems of a plant of each variety and after the two stems were brought together they were bound with raffia. Five days were allowed for the graft to take, when the Stringless Green Refugee plant was inoculated with the common mosaic strain of bean virus 1, by rubbing inoculum on the primary leaves with carborundum. After a period of 10 days, 17 of 24 success-

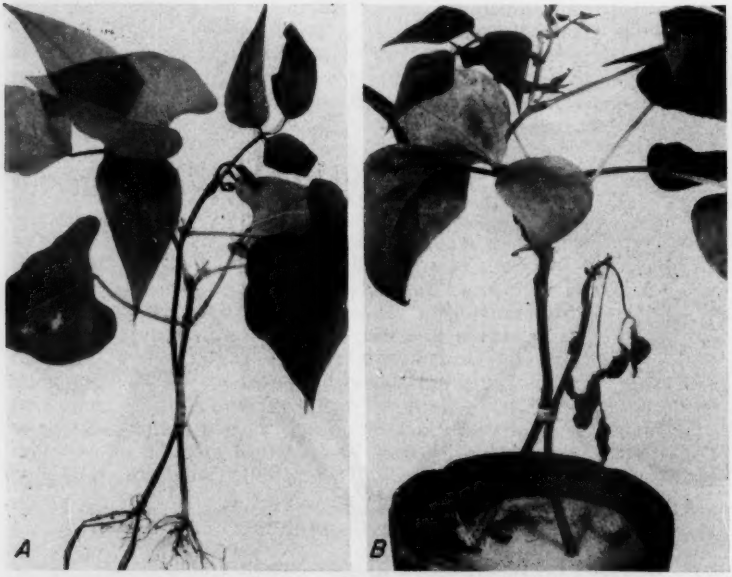

FIGURE 2.—Approach grafts between plants of Sensation Refugee 1066 and Stringless Green Refugee. *A* (right), Stringless Green Refugee plant inoculated with the common mosaic strain of bean virus 1; *A* (left) systemic necrosis in Sensation Refugee 1066 plant, resulting from approach graft. *B* (left), Stringless Green Refugee plant, inoculated with common mosaic, showing leaf curl typical of common mosaic; *B* (right), Sensation Refugee plant that has died from severe necrosis, as a result of inoculation with mosaic.

ful grafts in the Sensation Refugee 1066 plants showed systemic necrosis and after 20 days all necrotic plants were dead. In each of the 17 grafts where necrosis and death of the resistant plant occurred, symptoms typical of common mosaic appeared on the Stringless Green Refugee plants. Five grafts of Stringless Green Refugee plants that were not inoculated, remained healthy. The development of the disease following approach-graft inoculations is shown in figures 2 and 3.

In another experiment the plants of five grafts were allowed to grow until pods had developed, before the Stringless Green Refugee plants were inoculated. In three of the grafts the inoculated plants

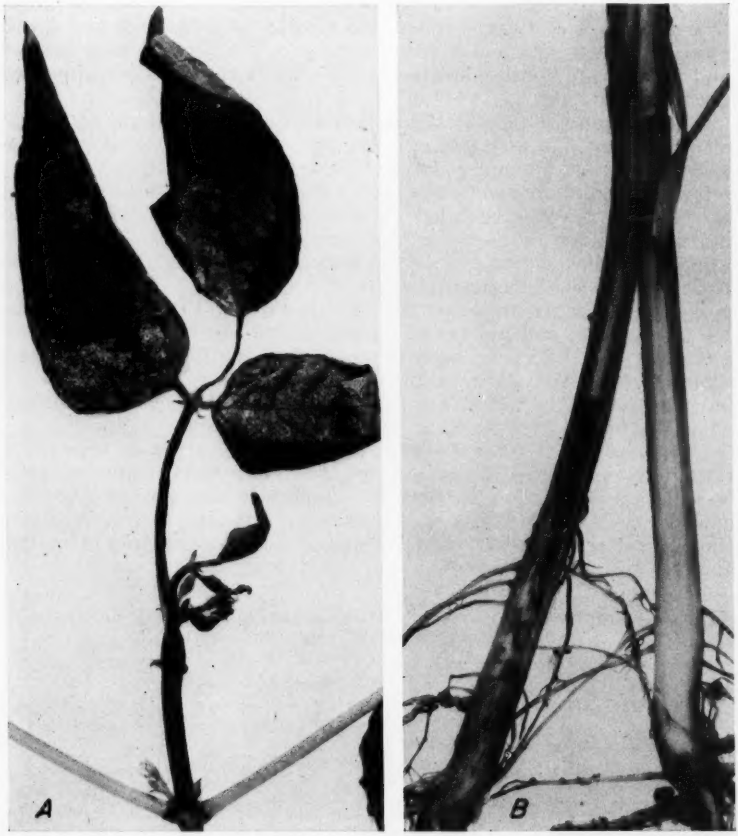

FIGURE 3.—*A*, necrosis of stem and leaves at growing point of plant of Sensation Refugee 1066 inoculated with common mosaic strain of bean virus 1, by the approach-graft method. *B* (right), lower portions of Stringless Green Refugee plant; and *B* (left), Sensation Refugee 1066 plant, inoculated by approach-graft method, with the epidermis peeled back to show typical black root necrosis resulting from mosaic inoculation.

showed typical common mosaic with no necrosis while in the corresponding resistant Sensation Refugee 1066 plants there developed a systemic necrosis which extended up the stem and petiole. In the pods and roots blackening of the vascular system became strikingly evident. When the disease reached this stage the symptoms coincided exactly with those described by Jenkins (*6*, *7*) for black root. When the pods showing black root symptoms were used as inoculum on Stringless Green Refugee plants, typical common mosaic developed. Necrosis in mature plants is shown in figures 4 and 5.

The approach-graft inoculations were next extended to other resistant Refugee varieties and to resistant varieties in the UI Great Northern and Robust groups. The common mosaic, greasy-pod, and Burkholder's strains of bean virus 1 were used. Stringless Green Refugee was used as the susceptible member of each graft and it was inoculated with one of the three viruses. The list of varieties used and their reaction to inoculation by each virus through approach grafts are given in table 1.

The Stringless Green Refugee plants which received the inoculum all showed mottle and leaf curl typical of common mosaic, and no necrosis. Giant Stringless Green Pod is susceptible to bean virus 1 but often shows only mild symptoms and was classed by Pierce (*10*). as "tolerant." It responded as a suceptible variety and showed mottle but no necrosis. Varieties carrying resistance derived from Corbett Refugee all reacted, as did Sensation Refugee 1066 and Idaho Refugee in earlier experiments, with severe necrosis. UI 81 developed no symptoms when inoculated with either of the three virus strains. UI 15, Robust, and Michelite, which are resistant to the common and greasy-pod strains of bean virus 1 but susceptible to Burkholder's strain of bean virus 1 developed typical mottle and leaf curl when inoculated with the last-mentioned strain and developed no symptoms when inoculated with either of the other two strains.

It was apparent from the experiments described up to this point that in varieties highly resistant to the three virus strains necrosis resembling black root developed. Moreover, when a variety susceptible to a given strain was inoculated through approach grafts there developed typical symptoms of the disease consisting of mottle and leaf curl without necrosis.

TABLE 1.—*Reaction of 11 varieties or strains of bean to approach-graft inoculation with 3 strains of bean virus*

Variety or strain	Reaction to virus indicated		
	Common mosaic	Greasy pod	Burkholder's
Sensation Refugee 1066	Necrosis	Necrosis	Necrosis.
Sensation Refugee 1071	do	do	Do.
Idaho Refugee	do	do	Do.
U. S. 5 Refugee	do	do	Do.
C–18 Refugee	do	do	Do.
C–19 Refugee	do	do	Do.
UI 81	No symptoms	No symptoms	No symptoms.
UI 15	do	do	Mottle.
Robust	do	do	Do.
Michelite	do	do	Do.
Giant Stringless Green Pod	Mottle	Mottle	Do.

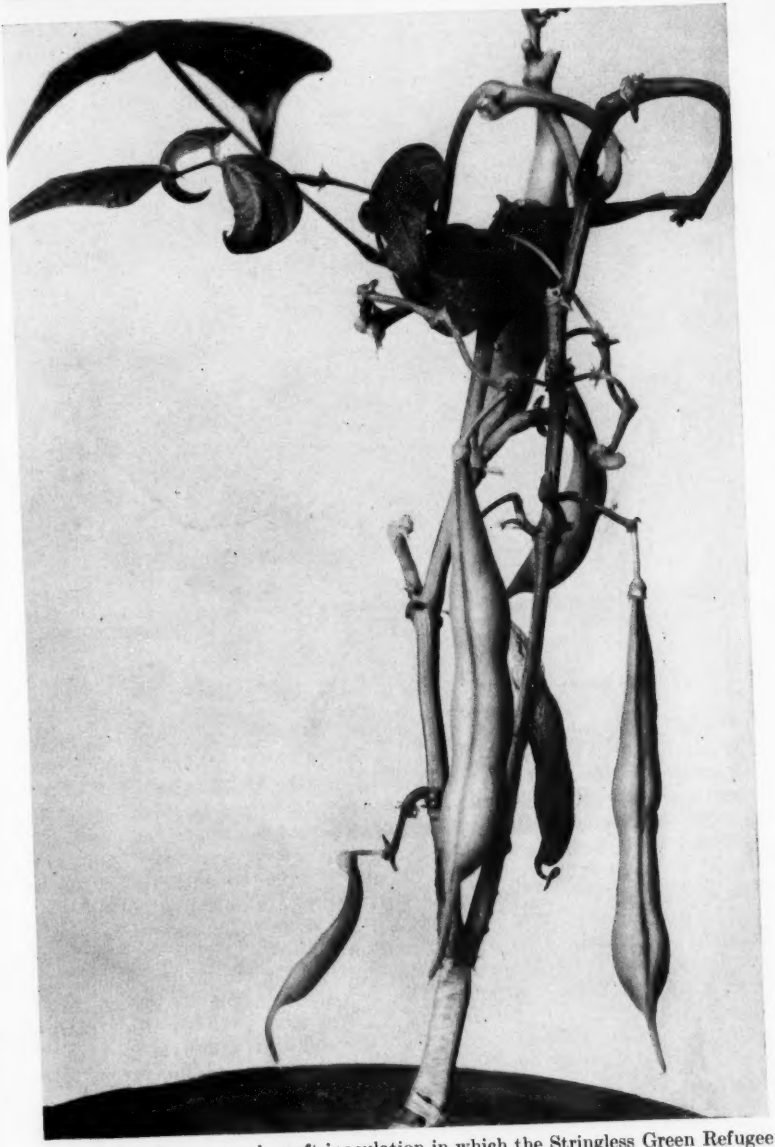

FIGURE 4.—An approach-graft inoculation in which the Stringless Green Refugee plant (left) was inoculated with bean virus 1 after pods were set. Leaf curl typical of common mosaic developed. On the other plant (Idaho Refugee) necrosis appeared on the stem and along the pod suture. See cross sections of pods in figure 5.

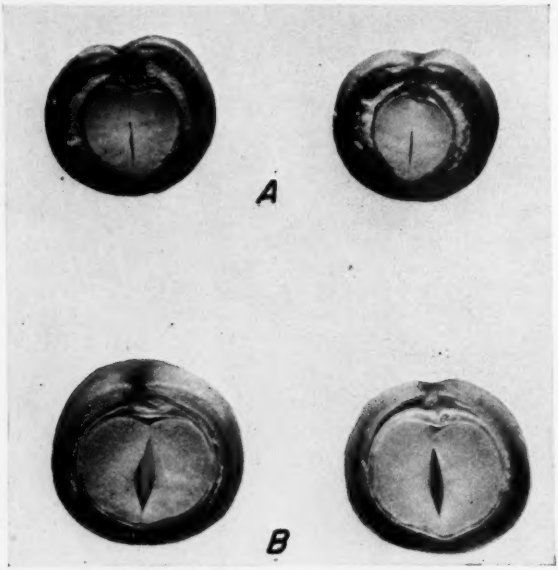

FIGURE 5.—Cross sections of pods from plants shown in figure 4. *A*, Pods of Idaho Refugee, showing necrosis typical of black root. *B*, Pods of Stringless Green Refugee, free from necrosis.

INOCULATION BY HYPODERMIC INJECTION

Twenty-seven varieties or strains of snap bean were observed during the summer of 1947 for the occurrence of hydrotic or greasy pods and for necrosis of the pods and roots (black root). Since varieties in which resistance was derived from Corbett Refugee had been observed in the greenhouse to show systemic necrosis when inoculated with the common mosaic strain of bean virus 1, there was a possibility that they might develop symptoms of black root if infected with this virus in the field. Jenkins (*6*) reported successful production of black root in the greenhouse by injecting the juice of necrotic pods into young healthy bean plants with a hypodermic needle. This method was employed to inoculate most of the varieties in the field. Inoculum was obtained from Stringless Black Valentine plants grown from infected seed in the greenhouse. Leaves and petioles from plants showing leaf mottle and curling were ground with a mortar and pestle and strained through two layers of cheesecloth. The inoculum, thus freed of bits of plant material which might clog the hypodermic needle, was injected into the petioles and stems of plants in the field after young pods were set. The plants from which inoculum was taken were kept in the greenhouse until maturity and were examined for the presence of necrotic pods and roots. None was observed on these plants or on other plants grown from the same lot of seed in the field.

In the first group were the varieties and strains derived from Corbett Refugee, which is resistant to the common mosaic strain of bean virus 1. The group contained Z–2, Z–4, Z–6, Z–8, Z–13, Z–22, Z–25, Florida Belle, C–18, C–19, Idaho Refugee, and Sensation Refugee 1071. Necrosis in a plant inoculated hypodermically with common mosaic is shown in figure 6. Within 2 or 3 days necrotic streaks

FIGURE 6.—A plant of Idaho Refugee showing extreme necrosis following inoculation with the common mosaic strain of bean virus 1, by hypodermic injection in a leaf petiole.

could be seen emanating from the point of inoculation, and within 9 days some of the plants showed streaking of stems and blackening of the pod sutures and the roots. About one-fourth of the plants inoculated hypodermically showed severe pod necrosis and black root. Some pods on plants in the advanced stages of necrosis showed hydrotic or greasy pods. When plants were inoculated by the rubbing method only, an occasional one showed necrotic pods. Plants of the variety Striped Creaseback reacted similarly to those in this group.

In the next group were the varieties Resistant Kentucky Wonder, Kentucky Wonder, Potomac, and McCaslan. These were thought to be susceptible to the common mosaic strain of bean virus 1 and were inoculated by the rubbing method. Several observations were made during the pod stage for greasy pods, but none was observed. Practically every plant showed blackening of the main veins of the pods, but upon breaking most of them did not show blackening of

the entire vascular system. Examination of the roots also showed a slight discoloration in the center, but no darkening in the cortical section such as occurred in the varieties with resistance derived from Corbett Refugee. However, an occasional plant was found in which the disease had advanced to a severe stage.

In the third group were varieties susceptible to the common mosaic strain of bean virus 1 but which were rated tolerant, as defined by Pierce (*10*). The varieties used were Giant Stringless Greenpod, Burpee Tenderpod, Burpee Stringless Greenpod, Plentiful, Stringless Black Valentine, Tendergreen, Bountiful, Keystonian, and Streamliner. After inoculation by rubbing, practically all plants showed hydrotic or greasy pod and leaf mottle or leaf distortion characteristic of common mosaic. Plants inoculated hypodermically after symptoms of common mosaic had developed did not develop necrosis of stems, pods, or roots.

Michelite was the only variety not in the above-mentioned groups. It is resistant to the common mosaic strain but susceptible to Burkholder's strain of bean virus 1. When inoculated hypodermically it did not show necrosis of pods or roots. This is in accord with the results of approach-graft inoculations in the greenhouse where varieties with the type of resistance of Robust showed no necrosis when inoculated with the common mosaic or the greasy-pod strain of bean virus 1.

GREENHOUSE STUDIES TO CHECK FIELD OBSERVATIONS MADE DURING THE SUMMER OF 1947

Since plants of the Potomac, Kentucky Wonder, Resistant Kentucky Wonder, McCaslan, and Striped Creaseback varieties developed symptoms of black root in the field it was necessary to determine their reaction to bean virus 1 under controlled conditions. Ten seeds of each of these varieties were planted in 6-inch pots in the greenhouse and, after the plants were in the 2-leaf stage, 5 plants of each variety were inoculated with the common mosaic virus by rubbing with carborundum. Five plants of each were left uninoculated to serve as controls. Ten days later these plants were examined for symptoms of common bean mosaic, but it was impossible to determine definitely whether or not they were infected since both inoculated and control plants had crinkled leaves but no definite mottle.

A composite inoculum was made from each group of five plants by taking one small trifoliate leaf from each plant and grinding them together. Five virus-free plants of Stringless Green Refugee were inoculated with each sample of composite inoculum. These plants were held for 25 days and examined for symptoms of common mosaic, but none was observed. This experiment indicates, therefore, that plants of these varieties are resistant to the common mosaic strain of bean virus 1. However, it should be pointed out that this applies to only one source of seed of these varieties and that plants from seed from other sources might react differently to inoculations with this strain of bean virus 1.

In order to test further the reaction of these varieties to bean virus 1, approach grafts were made to Stringless Green Refugee as previously described (Kentucky Wonder and Resistant Kentucky Wonder were not included because of lack of seed). Five days later

the Stringless Green Refugee portions of the grafts were inoculated with the common mosaic strain or the greasy-pod strain of bean virus 1. At the same time grafts of other varieties listed in table 2 were inoculated with either the common mosaic or the greasy-pod strain or with Burkholder's strain of bean virus 1 from the same source as described previously.

It is evident from the data presented in table 2 that at least some plants of McCaslan and Potomac express a necrotic reaction when infected with strains of bean virus 1. However, the few plants which did become necrotic had reached considerable size in contrast to plants of Sensation Refugee 1066 which were killed while still small. The reaction of Striped Creaseback was very similar to that of Sensation Refugee 1066.

TABLE 2.—*Reaction of several varieties to different graft combinations inoculated with 3 strains of bean virus 1*

Varieties grafted [1]	Virus used	Grafts tested	Plants showing necrosis [2]
		Number	*Number*
Robust	Greasy pod	3	0
Sensation Refugee 1066			
Do	Burkholder's	6	3
Michelite	do	10	10
Sensation Refugee 1066			
Stringless Green Refugee	Greasy pod	10	0
Robust			
Stringless Green Refugee	do	6	0
Michelite			
Do	Common bean mosaic	7	0
Stringless Green Refugee	do	17	8
Sensation Refugee 1066			
Do	Greasy pod	15	7
Stringless Green Refugee	Common bean mosaic	8	2
Striped Creaseback			
Do	Greasy pod	4	2
Stringless Green Refugee	do	10	1
McCaslan			
Stringless Green Refugee	do	8	1
Potomac			
Stringless Green Refugee	do	8	0
UI 15			
UI 15	Burkholder's	6	6
Sensation Refugee 1066			

[1] The first variety of each pair listed was inoculated mechanically.
[2] Necrosis in the plant in the graft that received the inoculum through the graft.

Plants of Sensation Refugee 1066 when grafted to Robust, Michelite, and UI 15, respectively, became necrotic when the last three varieties were inoculated with Burkholder's strain of bean virus 1. In contrast to this, when Robust was grafted to Sensation Refugee 1066 and inoculated with the greasy-pod strain, no symptoms developed in either part of the graft. This is to be expected since Robust is resistant to the greasy-pod virus. Robust, Michelite, and UI 15 are susceptible to Burkholder's strain, however; hence, when Sensation Refugee 1066 plants were inoculated with this virus, the typical necrotic reaction developed. There is no apparent difference in the reaction of Sensation Refugee 1066 plants to the common mosaic or greasy-pod strains, since each incited necrosis that was indistinguishable from that of the other.

When Robust, Michelite, and UI 15, respectively, were grafted to Stringless Green Refugee a mottle frequently developed in the first-

named varieties when the Stringless Green Refugee plants were inoculated with either the greasy-pod strain or the common mosaic strain. This is of interest because these varieties are reportedly resistant to these two viruses when inoculation is made by ordinary rubbing methods. It appeared that either the two viruses had become established through the graft or that the inoculum was a mixture of ordinary bean virus 1 and Burkholder's strain of this virus. In order to determine which explanation was valid, nine sample inoculations from these plants were made to five plants each of virus-free Michelite and Stringless Green Refugee, by the ordinary rubbing method. In two cases plants of Michelite and Stringless Green Refugee inoculated with virus from plants in which no mottle symptoms were evident did not develop mottle. However, inoculations from mottled plants produced mottle on both Stringless Green Refugee and Michelite that appeared to be identical with mottle produced by Burkholder's strain of bean virus 1. It therefore appears that the cultures of both the greasy-pod and the common mosaic viruses used were contaminated with Burkholder's strain of bean virus 1. However, the concentration of the contaminant was evidently at a low level, since no symptoms developed in Michelite or Robust when they were inoculated by ordinary rubbing methods.

EFFECT OF TEMPERATURE ON NECROSIS PRODUCED BY APPROACH-GRAFT INOCULATION

Grafts of Stringless Green Refugee and Sensation Refugee 1066 were prepared as previously described and held at 28° C. for 8 days before being inoculated. At the end of that time 32, 26, and 28 grafts were inoculated through Stringless Green Refugee with the common mosaic, greasy-pod, and Burkholder's strains, respectively. These viruses were obtained from the same cultures used in the experiment reported in the previous section.

Each virus-inoculated group was divided into four parts as nearly equal as possible (6 to 8 grafts) and placed in constant temperature houses maintained at 28°, 24°, 20°, and 16°, respectively. Seven days later the Sensation Refugee plants at 28° and 24° had developed a high percentage of necrosis with all three viruses, but only those inoculated with the common mosaic strain were necrotic at 20°. No symptoms had developed in any plants in the 16° house.

Fourteen days after inoculation most plants kept at 28° and 24° which had shown necrosis after 7 days were dead and a few of the plants inoculated with the common mosaic strain at 20° were also dead. Necrosis was just beginning to show in plants at 20° inoculated with the greasy-pod virus and in those inoculated with Burkholder's strain. A very low percentage of necrosis was developing in the plants at 16° inoculated with the common mosaic strain and the greasy-pod strain, and there was no necrosis in the plants inoculated with Burkholder's strain. After 28 days practically all plants at 28°, 24°, and 20° which were necrotic at earlier readings were dead. Plants inoculated with common mosaic strain and those inoculated with the greasy-pod strain at 16° had developed a high percentage of necrosis but no plants were dead.

Plants of Stringless Green Refugee inoculated with Burkholder's strain at 16° showed a very slight mottle, but there were no symptoms

in plants of Sensation Refugee 1066. Three of the Sensation Refugee plants 1066 moved from the 16° house to the 28° house developed necrosis after 5 days. This indicates that Burkholer's strain was present in the plants at 16° but did not produce necrosis at that temperature.

NECROSIS OF RESISTANT PLANTS CAUSED BY INSECT TRANSMISSION OF STRAINS OF BEAN VIRUS I

Jenkins (*6*) tested several species of insects as possible vectors of black root. He reported that transmission in one instance by the tarnished plant bug (*Lygus pratensis* (L.)) was doubtful but that no species of aphids were tried. Several workers (*8, 10, 17, 18*) have reported transmission of bean virus 1 by many species of aphids. Pierce and Hungerford (*12*) also reported successful transmission of this virus with aphids, but they were unable to transmit it with the tarnished plant bug.

The green peach aphid, (*Myzus persicae* (Sulz.)) was studied to determine whether it could transmit strains of bean virus 1 to plants with resistance derived from Corbett Refugee and thereby cause necrosis like that produced when inoculations were made by other methods. Nonviruliferous aphids were raised on healthy cabbage plants and later transferred to clean dry test tubes and allowed to starve for 3 hours. After starvation they were transferred to infected bean plants and allowed to feed for 20 to 25 minutes. At the end of that time, 10 to 15 aphids were transferred to each healthy plant that was to be inoculated. After feeding on these plants for 48 to 60 hours all aphids not already dead were killed by fumigation.

Bean virus 1 and two of its strains were transmitted to young Sensation Refugee 1066 plants by aphids. The incidence of systemic necrosis resulting was low. Two plants of 10 inoculated with Burkholder's strain, 1 of 13 inoculated with the greasy-pod strain, and 2 of 17 inoculated with the common mosaic strain developed systemic necrosis. When Stringless Green Refugee was treated in the same way, 9 of 13 plants developed systemic mottle and leaf curl. Several instances were noted in which necrosis had started in an isolated area of a leaf (presumably where an aphid had fed) but remained localized instead of spreading to become systemically necrotic, thereby causing death of the plant. Necrosis following aphid transmission of bean virus 1 to resistant plants, derived from Corbett Refugee, is shown in figures 7 and 8.

DISCUSSION

The results of this investigation show that the disease described as black root (*6*) is a symptom which results from the infection of certain varieties of bean by strains of bean virus 1. This hypersensitive reaction is most pronounced in those varieties that possess a high resistance derived from Corbett Refugee. Varieties in which resistance has been derived from Robust or Great Northern evidently possess a high degree of resistance or immunity to common bean mosaic, since they show no systemic necrosis in either young or old plants and do not develop black root symptoms. Other varieties that are tolerant of or susceptible to the common mosaic strain of

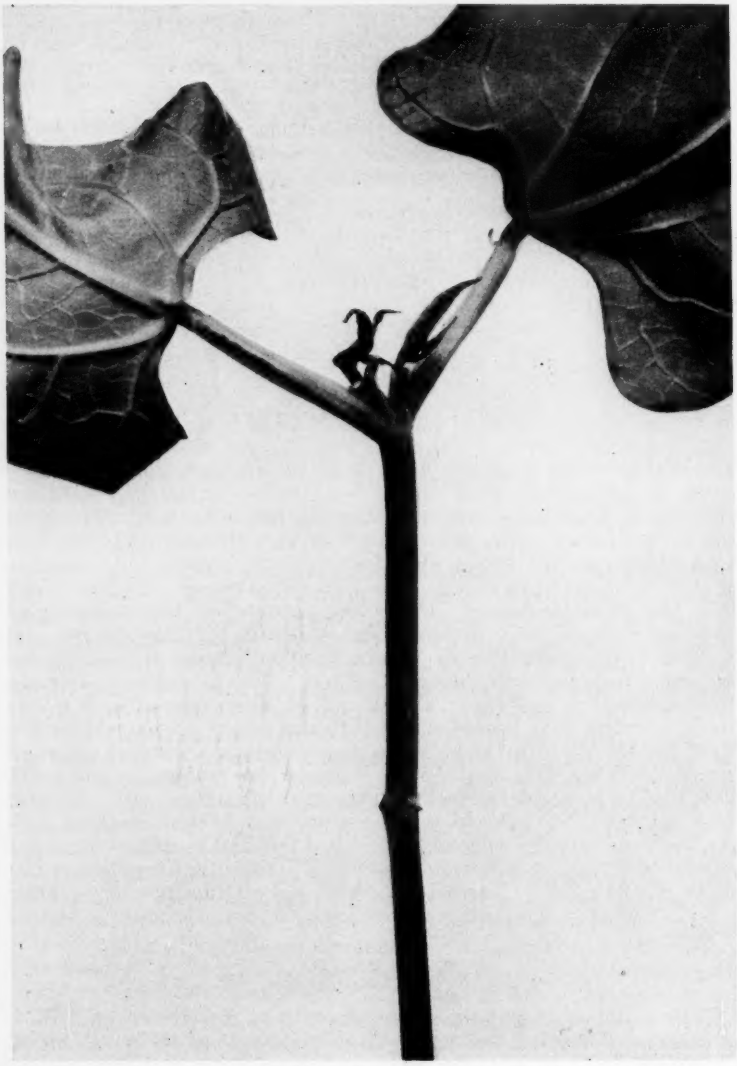

FIGURE 7.—Systemic necrosis in a plant of Sensation Refugee 1066 following aphid transmission of the common mosaic strain of bean virus 1. Note the unilateral development of stem necrosis and the veinlet necrosis in the corner of the leaf on the right.

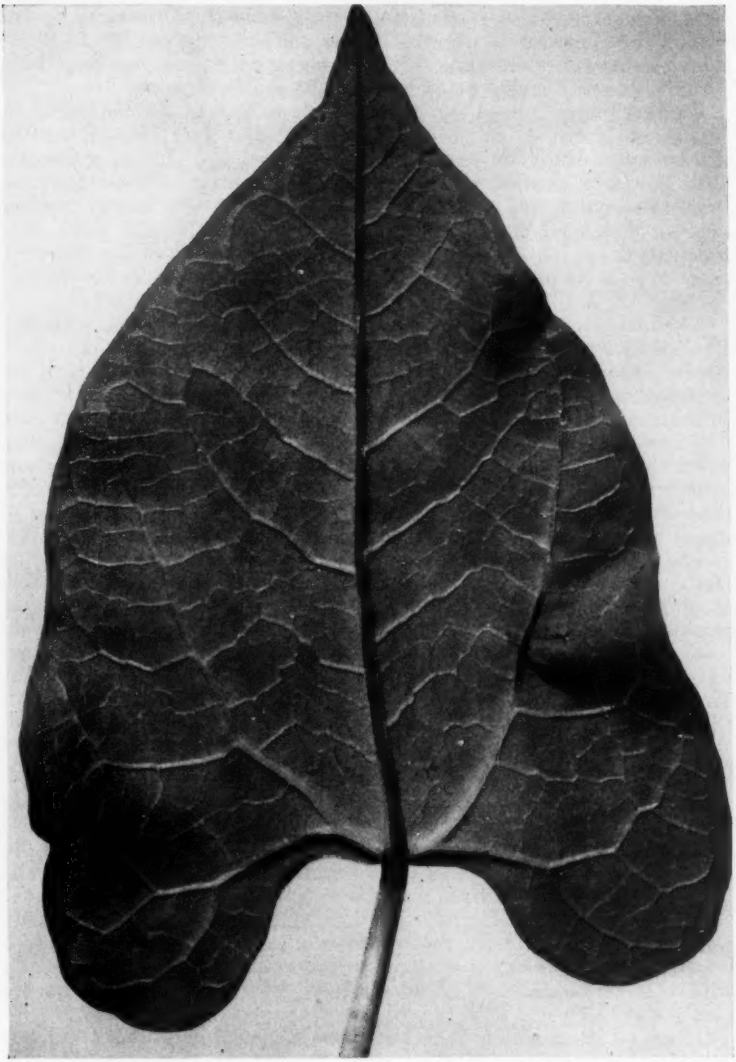

FIGURE 8.—Necrosis localized on a vein of Sensation Refugee 1066 following the feeding of an aphid which transmitted Burkholder's strain of bean virus 1.

bean virus 1 are also free of black root since their response to the virus is not necrotic in nature. Those varieties susceptible to Burkholder's strain but immune from the common mosaic strain of bean virus 1 do not develop black root for the same reason.

Jenkins found typical symptoms of black root in the field only on plants in bloom or in the young-pod stage. He transmitted the disease successfully by means of infected seed and therefore thought that the virus causing black root required a long incubation period before the symptoms appeared. This is not the case, however, since typical blackened pods have been observed on plants 9 days after inoculation, which is about the length of time required for the common mosaic virus to produce noticeable symptoms on susceptible varieties (*3*, *4*, *5*).

The incidence of black root is very low in all varieties that have the Corbett Refugee type of resistance. This is probably due in part to the fact that most plants of this type which become infected with the common mosaic strain develop systemic necrosis and die before pod formation. Seed transmission would therefore be at a minimum. The incidence of black root is extremely low in these varieties, even when they occur in fields adjacent to susceptible varieties that show a high percentage of bean mosaic. This is probably due to the fact that they possess "field resistance" which makes the incidence of insect transmission of the common mosaic strain very low.

The nature of this "field resistance" is not understood. Judging from observations of small localized necrotic areas in leaves on which viruliferous aphids had fed, it is possibly based on a hypersensitive reaction of the tissue to infection. In most cases this results in a small local necrotic area or fleck in which the virus is usually inactivated, while in a very few cases systemic necrosis and black root occur. When inoculation is made by approach graft or hypodermic injection the chances of a systemic relation of virus to host are greatly increased. When severe damage from systemic necrosis occurs the virus usually becomes inactivated in the early stages of disease development.

SUMMARY

This paper reports the results of an investigation to determine the reactions of bean varieties to three strains of bean virus 1 and to the typical strains of bean virus 2.

Varieties with the Corbett Refugee type of resistance previously reported as immune from the common mosaic strain of bean virus 1 were shown to develop a systemic necrosis and to die when infected with this virus and two of its strains. The symptoms on mature plants were identical with the disease described as black root.

Other varieties which have the Robust type of resistance did not develop this necrosis and were never observed to show symptoms of black root.

Varieties which are tolerant of or susceptible to the common mosaic strain were also free of necrosis and did not show symptoms of black root.

LITERATURE CITED

(1) ANDERSON, M. E.
1941. SENSATION REFUGEES, TWO NEW MOSAIC-RESISTANT VARIETIES. Canner 92 (7): 14–15, illus.

(2) BURKHOLDER, W. H.
1944. NEW VIRUS MAY THREATEN ROBUST PEA-BEAN IN NEW YORK. Farm. Res. [N. Y. State Sta.] 10 (1): 12, 16, illus.

(3) DEAN, L. L., and HUNGERFORD, C. W.
1946. A NEW BEAN MOSAIC IN IDAHO. (Phytopath. note) Phytopathology 36: 324–326.

(4) FAJARDO, T. G.
1930. STUDIES ON THE MOSAIC DISEASE OF THE BEAN (PHASEOLUS VULGARIS L.). Phytopathology 20: 469–494, illus.

(5) HARRISON, A. L.
1935. THE PHYSIOLOGY OF BEAN MOSAIC. N. Y. (State) Agr. Expt. Sta. Tech. Bul. 235, 48 pp., illus.

(6) JENKINS, W. A.
1940. A NEW VIRUS DISEASE OF SNAP BEANS. Jour. Agr. Res. 60: 279–288, illus.

(7) ———
1941. A HISTOLOGICAL STUDY OF SNAP BEAN TISSUES AFFECTED WITH BLACK ROOT. Jour. Agr. Res. 62: 683–690, illus.

(8) NELSON, R.
1922. TRANSFERENCE OF THE BEAN MOSAIC VIRUS BY MACROSIPHUM SOLANIFOLII.
Science 56: 342–344.

(9) PARKER, M. C.
1936. INHERITANCE OF RESISTANCE TO THE COMMON MOSAIC VIRUS IN THE BEAN. Jour. Agr. Res. 52: 895–915, illus.

(10) PIERCE, W. H.
1934. VIROSES OF THE BEAN. Phytopathology 24: 87–115, illus.

(11) ———
1935. THE INHERITANCE OF RESISTANCE TO COMMON BEAN MOSAIC IN FIELD AND GARDEN BEANS. Phytopathology 25: 875–883, illus.

(12) ——— and HUNGERFORD, C. W.
1929. SYMPTOMATOLOGY, TRANSMISSION, INFECTION AND CONTROL OF BEAN MOSAIC IN IDAHO. Idaho Agr. Expt. Sta. Res. Bul. 7, 37 pp., illus.

(13) ———, and WALKER, J. C.
1933. THE DEVELOPMENT OF MOSAIC-RESISTANT REFUGEE BEANS. Canner 77 (26): [7]–9.

(14) RICHARDS, B. L., and BURKHOLDER, W. H.
1943. A NEW MOSAIC DISEASE OF BEANS. Phytopathology 33: 1215–1216.

(15) WADE, B. L., and ZAUMEYER, W. J.
1938. U. S. NO. 5 REFUGEE, A NEW MOSAIC RESISTANT REFUGEE BEAN. U. S. Dept. Agr. Cir. 500, 12 pp., illus.

(16) WALKER, J. C., and JOLIVETTE, J. P.
1943. PRODUCTIVITY OF MOSAIC RESISTANT REFUGEE BEANS. Phytopathology 33: 778–788, illus.

(17) ZAUMEYER, W. J.
1933. TRANSMISSION OF BEAN MOSAIC VIRUS BY INSECTS. (Abstract.) Phytopathology 23: 40.

(18) ———, and KEARNS, C. W.
1936. THE RELATION OF APHIDS TO THE TRANSMISSION OF BEAN MOSAIC. Phytopathology 26: 614–629.

(19) ———, and THOMAS, H. R.
1947. GREASY POD—A NEW VIRUS DISEASE OF BEANS. (Abstract.) Phytopathology 37: 25.

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